Canadian Journal of Botany

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 30 JANUARY 1952 NUMBER 1

MICROFLORA AND THE HEATING OF DAMP STORED WHEAT[1]

BY NORMAN JAMES[2] AND A. R. LEJEUNE[3]

Abstract

Studies on damp wheat, bin-burned under conditions simulating natural storage and under adiabatically controlled conditions, produced evidence which provides the basis for the belief that: (1) heating and bin-burning of damp grain during storage are caused primarily by microorganisms; (2) many species of bacteria and of fungi, normally present on grain, are associated with this spoilage; (3) these species are mesophilic, rather than thermophilic; (4) under natural storage, although the grain shows evidence of increased combustion, most of the heat is dissipated. Consequently, the temperature does not rise sufficiently high for thermophiles; and (5) if the temperature in isolated pockets in the mass rises high enough, the thermophiles probably are the result rather than the cause of the high temperature.

Bin-burning or spontaneous heating has long been accepted as a major problem in the storage of grain at critical moisture levels. Until relatively recently, however, the part played by microorganisms in producing the heat was not recognized. Gilman and Barron (2) demonstrated that germination of disinfected wheat produced only a slight rise in temperature, compared to that of untreated wheat. Larmour, Clayton, and Wrenshall (4) treated experimental samples of wheat with carbon tetrachloride. Molds did not grow and the grain did not heat. In addition, embryo activity was lowered somewhat. Ramstad and Geddes (7) carried out extensive respiration studies on soybeans using adiabatic control equipment. These workers stated that, ". . . . . seemed to confirm the theory that microorganisms are the primary cause of the excessively high respiration rates responsible for heating . . . .". Sallans, Sinclair, and Larmour (8) using, in general, the same procedures as Ramstad and Geddes, but with flax seed and sunflower seed, concluded that heating of grains in commercial storage must result from active growth of the microflora rather than from embryonic activity. Leach (5) and Oxley (6), using a different approach to the problem and each employing a different technique, showed that removal of the embryo from the kernel did not reduce respiration appreciably. Leach noted that where the final moisture content of the wheat was not lower than 19% a distinct growth of fungal mycelium on

[1] *Manuscript received August 20, 1951.*

Contribution from the Department of Bacteriology and Animal Pathology, The University of Manitoba, Winnipeg, Man., with financial assistance from the National Research Council of Canada.

[2] *Professor of Bacteriology.*

[3] *Formerly Graduate Assistant in Bacteriology.*

[The December number of this Journal (Can. J. Botany, 29 : 585-633. 1951) was issued January 4, 1952.]

the grain was visible to the naked eye. Oxley reported finding a considerable fungal mycelium on the inner surface of the stripped epidermis of the wheat kernel.

The following studies, based on the approach of the bacteriologist, were conducted to provide information on the relationship between microorganisms on wheat and self-heating.

Microflora on Damp Wheat Heated under Commercial Storage

Two experiments were conducted to obtain information on the microflora of damp wheat heated under conditions simulating normal storage. For Expt. 1 the sample was obtained from a commercial elevator where the grain had been stored about eight months. It was selected because of its moisture (18.5%) and because previously, on account of the warm weather, it had been under close observation for evidence of heating. The sample was held in an airtight container in a 25° C. incubator. It was plated for bacteria and for fungi, by the method outlined previously (3), at 10 days and again at 56 days. At the latter time the sample showed evidence of typical damp-grain-heating damage,—dull and darkened kernels, slightly matted together by fungal mycelium. The counts are presented in Table I. At each plating the sample harbored exceedingly large numbers of mesophilic bacteria; but only small numbers of thermophilic bacteria. At 10 days mesophilic fungi were present in small numbers, but at 56 days their numbers were very large. At each plating, thermophilic fungi did not develop on any of the plates prepared. Both the bacterial and fungal populations were heterogeneous, as had been found previously on unheated commercial samples (3). Consequently, detailed study of types was not undertaken.

TABLE I

NUMBERS OF MICROORGANISMS ISOLATED AT THREE INCUBATION TEMPERATURES FROM DAMP WHEAT HELD AT 25° C., BACTERIA PER GM. ON NUTRIENT AGAR AND FUNGI ON CZAPEK'S AGAR

Days wheat held	Type of organism	Incubation in °C.		
		23°	37°	50°
10	Bacteria	48,000,000	18,000,000	500
56	Bacteria	27,000,000	5,000,000	7000
10	Fungi	22,000	100	0*
56	Fungi	20,000,000	1,270,000	0

* *No colonies on plates prepared from the 1 : 10 dilution, nor on any of the higher dilutions.*

The sample for Expt. 2 was an experimental plot sample, which had been held in the laboratory for 20 months. It was dry and brittle and harbored a small population. Two aliquots were used, one conditioned to 22.9% and the other to 19.3% moisture. Conditioning was accomplished by adding the calculated amount of water needed, mixing on a mechanical mixer for 30 min.,

and refrigerating at about 9° C. for three days. Each aliquot was placed in a quart Dewar flask, fitted with an aeration device and a thermometer. About one liter of air was forced through each sample daily. It was introduced near the bottom of the grain and escaped through a small opening in the cork. Air flow was maintained by dripping water at a controlled rate into a sealed 15 liter flask, from which the displaced air passed through tubing into the grain. The humidity of the air was adjusted to equilibrium with the moisture of the wheat (Coleman and Fellows (1)) by passing the air through a sulphuric acid solution of predetermined strength, according to the method of Wilson (9). The experiment was carried out in a room held at 23° C. (±1°). Starting at six days the temperature of the aliquot with 22.9% moisture rose above that of the room, exceeding it by about 4° C. at the highest, whereas the temperature of the aliquot with 19.3% moisture remained at that of the room. At 25 days both aliquots showed evidence of heat damage. Consequently, the experiment was terminated. In both aliquots, the moisture had lowered slightly, to 21.5 and 18.0%, respectively; and germination to 8 and 29%, respectively. In both aliquots fungi developed on plates for fungi incubated at 23° C., and on those for bacteria as well, to such an extent that study of numbers and types was not possible. On plates incubated at 37° C., likewise in both aliquots, fungi predominated, although numbers were smaller than on plates incubated at 23° C. However, on plates incubated at 50° C. and in both aliquots neither bacteria nor fungi developed.

Microflora on Damp Wheat Heated under Adiabatic Conditions

The adiabatic equipment used in this study was essentially the same as that used by Ramstad and Geddes (7), and by Sallans *et al.* (8). Obviously in this type of study, the validity of heat-production data is dependent on the control unit keeping the temperature of the chamber very close to, but lower than, that of the sample. If the temperature of the chamber were even slightly higher than that of the sample, the rise in temperature of the sample would result from external heat and would have no significance in the problem under study. If, on the contrary, the temperature of the chamber were not held close to that of the grain, the rise in temperature of the grain would be too low because of dissipation of heat. The equipment was adjusted using water at about 40° C. in the flask, until the control would permit a drop of 0.1° C. per day. This sometimes took as long as a week. Even so, there was no assurance that the adjustment was satisfactory for every temperature level from 25° to 65° C. Nor could it be certain that the control would respond exactly the same when reassembled after the water was replaced by wheat, although care was exercised in replacing the thermopile in the proper position. As a practical check on the operation of the heat-control unit during an experiment, a sample of air-dried wheat was placed in a second Dewar flask in the chamber. A thermometer was inserted into it. If the temperature of the moist grain rose above that of the dry grain the control was working properly, although this gave no indication of sensitivity.

The samples* used in the adiabatic chamber experiments were grown and harvested on an experimental plot basis. They represented high quality hard spring wheats—sound, dry, relatively free from extraneous matter, and 100% viable. In preparation for an experiment the sample was tested for moisture and for germination, and plated for bacteriological studies. At the same time the control equipment was standardized. The sample was then conditioned to a favorable moisture for heating, as outlined in the previous section, and finally tempered to a temperature slightly above that of the room where the adiabatic chamber was located. The tempering served two purposes. A temperature difference was necessary to operate the control unit from the beginning; and the higher temperature favored microorganic activity. The temperature data recorded on four experiments are presented in Table II.

TABLE II

TEMPERATURE OF DAMP WHEAT SPONTANEOUSLY HEATING UNDER ADIABATIC CONTROL, DEGREES C.

Day	Experiment			
	3	4	5	6
0	26.5	30.3	27.7	29.3
1	27.3	31.5	29.5	32.3
2	27.7	31.8	32.5	34.7
3	28.4	32.1	36.0	38.5
4	29.3	33.3	41.9	46.2
5	30.6	34.3	47.6	53.5
6	31.9	34.5	54.2	56.2
7	33.5	35.9	56.1	55.2
8	34.7	—	56.7	52.0**
9	35.8	39.2	60.3	54.4
10	37.0	40.8	62.8	57.6
11	38.2	42.3	64.0	60.5
12	39.5	43.7	42.1**	61.3
13	40.9	44.6		61.7
14	42.4	45.2		62.1
15	43.9	45.9		65.1
16	45.3	45.7		67.7
17	46.0	46.1		65.8
18	46.5	46.5		
19	46.8	47.0		
20	47.0	47.1		
21	47.0	——		
22	47.0	75.0*		
23	47.0			
24	47.0			
25	46.7			
26	41.1			

— *Reading not taken.*

* *Relay stuck, external heating.*

** *Failure of power or light source, heat dissipation.*

In each case the temperature rose, rapidly in the first week in Expts. 5 and 6, and gradually and not so high in Expts. 3 and 4. Expts. 3 and 6 were terminated deliberately after the peak temperature was reached. Expts. 4 and 5

* *Courtesy of Dominion Laboratory of Cereal Breeding, Winnipeg, Man.*

were terminated because of failure of the control unit after having operated normally up to the time of failure. In each case the sample removed from the adiabatic chamber showed typical evidence of bin-burning. It was plated within a few hours after removal from the chamber, the same procedures being used as on the unheated sample or the control plated at the beginning of the experiment. The results are presented in Table III. The difference in count

TABLE III

EFFECT OF SPONTANEOUS HEATING UNDER ADIABATIC CONTROL ON NUMBER OF BACTERIA AND FUNGI ON DAMP WHEAT

Expt.	% Moisture	Incubation temp., °C.	Bacteria per gm.		Fungi per gm.	
			Control*	Heated*	Control	Heated
3	19.17	23	190,000	0**	180	46,000
		37	26,000	0	0	37,000
		50	0	0	0	4300
4	21.77	23	370	0	30	460,000
		37	100	0	10	1,000,000
		50	0	0	0	200,000
5	23.50	23	400	0	0	0
		37	100	0	0	0
		50	20	7,200,000	0	0
6	22.23	23	80	160,000	0	0
		37	60	7,000,000	10	0
		50	80	4,800,000	0	0

* *Control sample plated before grain was introduced to adiabatic chamber; heated sample, after experiment terminated.*

** *No colonies on plates prepared from the 1 : 10 dilution, nor on any of the higher dilutions.*

was greatest with fungi in Expts. 3 and 4 and with bacteria in Expts. 5 and 6. In contrast to what had been found in Expts. 1 and 2 with samples showing evidence of bin-burning, but with heat dissipation, a large population developed on plates incubated at 50° C. in each of the adiabatic-chamber experiments. Likewise in contrast, the population developing on these plates was homogeneous or, at most, consisted of two species. However, it differed in the different experiments: in Expt. 3, *Penicillium melinii* Thom and *P. viridedorsum* Biourge; in Expt. 4, *Mucor* sp. and *Aspergillus fumigatus* Fesenius; and in Expts. 5 and 6, a species of *Streptomyces* Waksman and Henrici. Expt. 6 was conducted on a replicate of the sample used for Expt. 5; otherwise the samples used were different.

Cause Versus Effect in the Microflora-heating Relationship

The foregoing results show a relationship between increase in certain species of microorganisms and increase in temperature. However, they fail to establish which is the precursor in the relationship. To obtain information on this

problem Expt. 7 was set up on the same basis as Expts. 3–6 inclusive, with the following addition. Four separate 50-gm. aliquots of the sample* used in the Dewar flask were placed in the adiabatic chamber, near the Dewar flask with the control unit. These samples were removed, one at a time, at appropriate intervals during the heating (care being taken not to interfere with the control equipment) and immediately plated. This gave six sets of plating data in the one experiment, each representing a stage in the heating process. The results are presented in Table IV. Those representing early stages of heating were

TABLE IV

NUMBERS OF MICROORGANISMS AT DIFFERENT STAGES IN THE SPONTANEOUS HEATING OF DAMP WHEAT UNDER ADIABATIC CONTROL, BACTERIA PER GM. ON NUTRIENT AGAR AND FUNGI ON CZAPEK'S AGAR

Incubation of cultures, temp., °C.	Days					
	0	2	4	6	11	17
	Temp. wheat, °C.					
	29.3°	34.7°	46.2°	56.2°	61.3°	65.8°
Bacteria						
23°	80	12,000	19,000,000	13,300,000	13,500,000	16,000
37°	60	16,000	17,800,000	14,700,000	**	7,000,000
50°	80	530	**	**	**	4,800,000
Fungi						
23°	0*	9000	240,000	1,500	320	50
37°	10	14,000	300,000	200	60	0*
50°	0*	400	140,000	0*	0*	0*

* *No colonies on 1 : 10 dilution plates, nor on any of the higher dilutions.*
** *Too many colonies for counting on 1 : 100,000 dilution plates.*

strikingly different from those obtained when the temperature had reached a high level. The population was large and represented many species. This population became smaller as the temperature increased and evidently was not present in the sample removed from the Dewar flask after 17 days in the chamber. The population in the final sample consisted of only a few species, thus verifying the finding on the heated samples in Expts. 3 – 6. Strangely, the population developing on nutrient agar at 50° C. was distinctly different

* *Same wheat as was used in Expts. 5 and 6.*

on the $\times 10^5$ plates from that on the $\times 10^4$ plates. On the $\times 10^5$ plates the same species of *Streptomyces* developed as had been encountered in Expts. 5 and 6; while on the $\times 10^4$ plates a *Bacillus* sp. was present. This latter species had been prominent on plates prepared from samples after 4, 6, and 11 days. This paradox may be explained on the basis of antagonism, or probably competition, or both.

This experiment was repeated with another sample of the same wheat, with essentially the same results.

Discussion

Infallible evidence that microorganisms are responsible for the heating of damp grain could be produced only if a check sample free of all microorganisms were available, which is probably beyond the realm of practicability. The evidence would involve not only the use of such a sample, but the added problem of keeping it free from contamination during the course of the experiment. That type of evidence has not been presented. However, the evidence presented is rather convincing. The population was small in the preheated sample in every case where such a control was available and exceedingly large in every heated sample (Table III). The population increase was most rapid during the period of most rapid temperature increase. The population rose in the first four days from practically nothing to numbers that would disqualify most perishable foods. At the same time the temperature rose 17° C. (Table IV). The population was large in every sample spoiled under more practical conditions where heat could be dissipated (Expts. 1 and 2).

The population responsible for the damp-wheat-heat damage under practical conditions undoubtedly consists of bacteria and fungi of many species that might be considered normal soil types, mesophilic rather than thermophilic species. The population developing at 50° C. was relatively small in Expts. 1 and 2, and the mesophilic population was large during the period of most rapid temperature rise in Expt. 7 (Table IV).

Data from the adiabatically controlled experiments appear to justify the belief that, as the temperature rises above that at which the mesophiles can exist, there is a change in the flora most actively proliferating and releasing heat. If a sample were tested as soon as it reached 50° C., a number of species would be encountered. However, if it were feasible to hold the sample at 50° C. for 10 days without further rise in temperature, it is highly probable that if tested then only a single species (or at most only a few species) would be encountered and it would be truly thermophilic. The others would have disappeared because of an unfavorable environment. That could explain the presence of the small number of species on the wheat at the end of prolonged heating under adiabatic control. This change in flora as the temperature rises likewise could account for the difference in daily temperature increment. In Expt. 6 (Table II) the average daily temperature increment from Day 3 to Day 5 was $7\frac{1}{2}$° C.; from Day 11 to Day 14, $\frac{1}{2}$° C.; and from Day 14 to Day 16, $2\frac{8}{10}$° C.

Any attempt to relate the finding of a specific thermophile in any of these samples to anything of practical value, on the basis of present knowledge, would be futile. Because of the accepted ubiquity of microorganisms, it is highly probable that the species found on one sample by this enrichment process under adiabatic control would be found on any other sample studied, if the exact environmental conditions could be duplicated. Likewise, it is highly improbable that under natural heating in an elevator or in a farm granary similar environmental conditions could prevail.

References

1. COLEMAN, D. A. and FELLOWS, H. C. Hygroscopic moisture of cereal grains and flaxseed exposed to atmospheres of different humidity. Cereal Chem. 2 : 275-287. 1925.
2. GILMAN, J. C. and BARRON, C. H. Effect of molds on temperature of stored grain. Plant Physiol. 5 : 565-573. 1930.
3. JAMES, N., WILSON, J., and STARK, E. The microflora of stored wheat. Can. J. Research, C, 24 : 224-233. 1946.
4. LARMOUR, R. K., CLAYTON, J. S., and WRENSHALL, C. L. A study of the respiration and heating of damp wheat. Can. J. Research, 12 : 627-645. 1935.
5. LEACH, W. Studies on the metabolism of cereal grains. III. The influence of atmospheric humidity and mould infection on the carbon dioxide output of wheat. Can. J. Research, C, 22 : 150:161. 1944.
6. OXLEY, T. A. The spontaneous heating of stored cereals. Sci. J. Roy. Coll. Sci. 15 : 71-80. 1945.
7. RAMSTAD, P. E. and GEDDES, W. F. The respiration and storage behaviour of soybeans. Univ. Minn. Agr. Expt. Sta., Tech. Bull. 156. 1942.
8. SALLANS, H. R., SINCLAIR, G. D., and LARMOUR, R. K. The spontaneous heating of flaxseed and sunflower seed stored under adiabatic conditions. Can. J. Research, F, 22 : 181-190. 1944.
9. WILSON, R. E. Humidity control by means of sulphuric acid solution, with critical compilation of vapour pressure data. Ind. Eng. Chem. 13 : 326-331. 1921.

PRODUCTION AND PROPERTIES OF A POLYPHENOL OXIDASE FROM THE FUNGUS *POLYPORUS VERSICOLOR*[1]

By W. M. Dion[2]

Abstract

The fungus *Polyporus versicolor* was shown to produce an extracellular polyphenol oxidase system during growth in submerged culture. The enzyme was stable and active in acid solution, and ozidized lignin, vanillin, ferulic acid, and other phenolic substances. Vanillin and ferulic acid were converted to new compounds as a result of enzyme action, whereas lignin absorbed oxygen and appeared to become more water soluble.

Introduction

The mechanisms by which lignin is synthesized and degraded have attracted increasing attention during recent years. Lignin is comparatively resistant to decay, and though it has been known for years that many fungi are able to attack it in fresh or decomposed plant materials, it is only comparatively recently that critical work on the degradation of lignin by fungi has been carried out. Researches such as those by Ledingham and Adams (5) and Day, Pelczar, and Gottlieb (2, 6) etc. are typical of the trend. Fungi have been found that are capable of growing on a synthetic medium containing isolated native lignin as the sole source of carbon (6) and an enzyme capable of oxidizing native lignin has been isolated from mushroom spawn (3).

The following report is a preliminary description of an extracellular enzyme produced by the mold *Polyporus versicolor*, which oxidizes lignin and attacks vanillin, ferulic acid, and a wide range of other simple phenolic substances.

Cultural Studies

Two strains, PRL 572 and 573, of the white rot of wood fungus, *Polyporus versicolor* L. ex Fries, were obtained from Dr. Nobles, of the Department of Agriculture, Ottawa.

An attempt was made to grow these strains on the medium used by Pelczar *et al.* (6) using indulin, a commercial preparation of pine-wood lignin, as the only source of carbon. Growth, however, was slow and slight on this medium in both surface and submerged culture. Since an adequate supply of native lignin was not immediately available, growth on other substrates was investigated. It was found that both isolates of the fungus grew rapidly and well in submerged culture on a medium consisting of minerals, glucose, and yeast extract. After 8 to 10 days' growth, the culture liquid was freed from cellular material and found to contain an enzyme which produced very pronounced

[1] *Manuscript received August 7, 1951.*
Contribution from the National Research Council, Prairie Regional Laboratory, Saskatoon, Sask. Issued as Paper No. 131 on the Uses of Plant Products and as N.R.C. No. 2609

[2] *Mycologist.*

color changes when added to solutions of indulin or other phenols. This enzyme was also produced when casein, haemoglobin, or peptone were used as nitrogen sources. However, when inorganic nitrogen sources, such as sodium nitrate, ammonium sulphate, or ammonium nitrate were used, no enzyme was detected. This may have been due to the very poor growth made with the inorganic nitrogen sources. The fungus made little or no growth on the medium of Pelczar *et al.* when native aspen lignin, wheat-straw lignin, indulin, vanillin, or ferulic acid were provided as carbon sources, as long as an inorganic nitrogen source was used. When an organic nitrogen supply was provided, growth was fair on these substrates, but did not compare with that produced when glucose was the carbon source.

The medium finally chosen for the production of the enzyme preparation used in these studies was as follows:

Glucose	30	gm.
Yeast extract	10	gm.
KH_2PO_4	1.0	gm.
NaCl	0.5	gm.
$MgSO_4.7H_2O$	0.5	gm.
$FeSO_4.7H_2O$	0.01	gm.
$ZnSO_4$	0.01	gm.
$CaCO_3$	1.0	gm.
Distilled water	1000	ml.

No studies were made on the effect of the various mineral salts on enzyme production. One-hundred milliliter aliquots of the above medium were autoclaved in 500 ml. Erlenmeyer flasks, and inoculated with 2 ml. aliquots of a mycelial suspension of PRL 572 blended in water. The flask cultures were incubated for 10 days at 30° C. on a Gump rotary shaker with a 1 in. radius of motion, operating at 225 r.p.m. After growth, the contents of the flasks were filtered, and the culture liquid stored at 5° C. under toluol. The culture medium after growth had a slightly acid reaction, usually between pH 4.5 – 5.0, in which range the enzyme appeared to be quite stable when kept at 5° C.

Both isolates of *P. versicolor* produced abundant yields of enzyme when grown on the above medium, and one isolate of *Armillaria mellea* PRL 586 produced low yields of what appeared to be a similar enzyme. However, growth of this latter fungus even in submerged culture, was so slow that no further work was done with it.

Methods of Estimating Enzyme Activity

Since it is well known that certain wood-destroying fungi, particularly the white rots, produce oxidizing enzymes that cause marked color changes in a number of phenolic substances, the enzyme from PRL 572 was first tested for visual color changes when added to a range of phenolic materials. These tests were carried out at 37° C. by the addition of 1 ml. of culture filtrate to

5 ml. of a 0.1% solution of substrate in unbuffered solution at pH 4.90. Controls were used in all cases using boiled enzyme.

The main interest in this enzyme, or enzyme system, was in its effect upon lignin. However, the lignin preparations used, with the exception of indulin, were insoluble in acid solution in the range where the enzyme showed maximum activity. For this reason vanillin and ferulic acid, two simple phenols related to lignin, were used for the preliminary tests. Both these substances would remain in solution in high dilutions down to a pH of about 4.50. Enzyme activity was estimated by measuring the rate of change of the ultraviolet absorption spectrum at appropriate wave lengths. Owing to the extreme sensitivity of this method, very dilute solutions were employed. The substrate concentration was usually 0.0016%, but varied slightly with the different substances used. Twenty-five milliliters of substrate solution containing 10 ml. of McIlvaine's disodium phosphate – citric acid buffer at pH 4.80 were brought to 37° C. and 0.5 ml. of enzyme solution added. Aliquots of 4 ml. were taken at intervals and the change in optical density at certain predetermined wave lengths read on a spectrophotometer.

Oxygen absorption was measured with a Warburg apparatus. One milliliter of water containing 15 mgm. of substrate was placed in a Warburg vessel of 20 ml. capacity. One-half of a milliliter of buffered enzyme solution was placed in the side arm. The disodium phosphate – citric acid buffer was adjusted so that the final concentration in the flask would be 0.025 *M* at pH 4.85. One-fifth of a milliliter of 10% potassium hydroxide was added to the center well. The vessels and their contents were brought to 37° C. and the enzyme added to the substrate from the side arm. The enzyme itself had a small endogenous absorption of oxygen which was corrected for.

Lignin Preparations

Three different types of lignin were used in this work.

1. "*Native*" *aspen lignin.*—This was prepared by Braun's procedure and the sample was obtained from Dr. J. M. Pepper of the University of Saskatchewan.

2. *Wheat straw lignin.*—This was obtained by refluxing the straw with a 1 : 1 dioxane – water solution made alkaline with 2% sodium hydroxide followed by precipitation with acid and washing. This lignin is soluble in many solvents and was obtained from Dr. J. E. Stone of this laboratory.

3. *Indulin A.*—A purified pine-wood lignin made by the West Virginia Pulp and Paper Company. Indulin is soluble in alkali and in dilute solutions will remain in solution at pH values lower than 5.0.

Results

Visible Reactions

Table I gives a list of color changes brought about by enzyme action. These reactions were prevented or delayed when the solutions were made alkaline.

TABLE I

COLOR CHANGES CAUSED BY AN ENZYME FROM PRL 572, ACTING ON PHENOLIC SUBSTRATES

Substrate	Visible reaction	Rate
"Native" aspen lignin	None	—
Wheat straw lignin	None	—
Indulin	Reddish purple	Rapid
Conidendrin	Brown ppt.	Rapid
l-Syringylpropene	Orange	Moderate
β-Syringylethanol	Light red-brown	Slow
Syringaldehyde	Light red-brown	Rapid
Vanillin	Brown-gray ppt.	Moderate
5-Bromovanillin	Milky	Rapid
5-Nitrovanillin	None	—
Ferulic acid	Orange ppt.	Rapid
Guaiacol	Red-brown, cloudy	Rapid
Anethole	None	—
Phenol	Dark red-brown	Slow
p-Cresol	Cloudy, later yellow	Slow
Resorcinol	Straw colored	Moderate
Catechol	Dark brown	Rapid
Tyrosine	Straw colored	Slow
Hydroquinone	Pale red-brown	Slow
Quinone	Red brown*	Slow

* *Control solution also turned the same color.*

The change in color of these solutions would indicate that *ortho-* or *para*-quinone formation has taken place, as is common with phenolic solutions in the presence of an oxidizing agent. However, when the 5-position is blocked, as in the case of syringaldehyde, only the formation of a *para*-quinone is possible. This will result in a reaction similar to the Dakin reaction (1) when the action of caustic soda and peroxides on *para*-substituted phenols will oxidize the aldehyde group to an OH group resulting in a reddish solution. These reagents will oxidize 5-bromo- but not 5-nitrovanillin, which is also the case with the enzyme produced by PRL 572. The structure of β-syringylethanol eliminates the formation of an *ortho*-quinone, and also a reaction of the Dakin type, unless the side group is oxidized to an OH group. Lack of any visible color change with anethole seems to indicate the need for a free OH group.

pH Optimum for Enzyme Activity

Preliminary tests had shown that the enzyme system from PRL 572 was stable and active only in neutral and acid solutions. Measurements of the rate of darkening of indulin solutions, and the decrease in density of the ultraviolet spectrum of ferulic acid at 287 mμ, showed that in both cases the maximum activity of the enzyme was about pH 4.80, and that the enzyme was almost completely inactive above pH 6.50. These results are given in Figs. 1 and 2.

The color change of indulin was measured by recording the time taken to reach the end point, which was estimated visually, and was thus subject to considerable personal error. However, results could be duplicated with fair

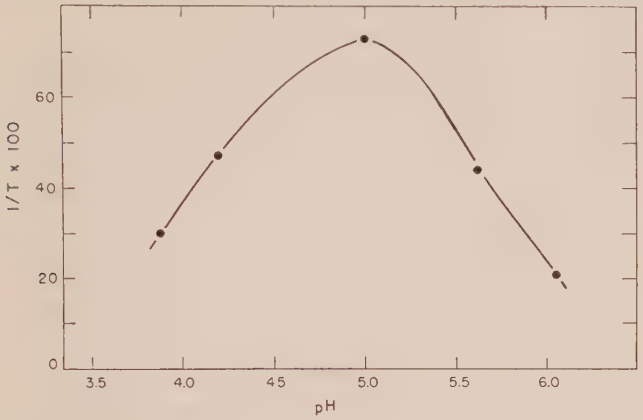


FIG. 1. The effect of pH on the rate of color change of indulin in the presence of a polyphenol oxidase preparation from the fungus *P. versicolor*.
T = time taken to reach the standard color-endpoint.

accuracy. The change in ferulic acid was measured by determining the change in optical density at a wave length of 250 mμ. Aliquots of the reaction mixture were withdrawn at intervals and read on the spectrophotometer against a blank of buffer and enzyme. After plotting the curves for each

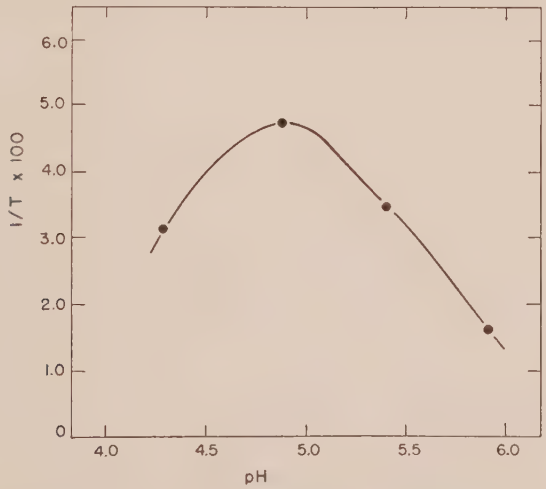


FIG. 2. The effect of pH on the rate of change in optical density of ferulic acid in the presence of a polyphenol oxidase preparation from the fungus *P. versicolor*, measured at a wave length of 287 mμ.
Reaction mixture, 25 ml. 0.00157% ferulic acid containing 10 ml. McIlvaine's buffer + 0.5 ml. culture filtrate diluted 1 : 10 with water incubated at 37° C.
T = time taken to reach a density of 0.600.

pH value, the time taken for the solution to reach a density of 0.600 was read and taken as a measure of enzyme activity. Solutions buffered at values above pH 6.50 did not approach this end point within a reasonable time, that is, within two or three hours.

Effect of the Enzyme on Ferulic Acid and Vanillin

The enzyme showed a remarkable affinity for ferulic acid and the culture filtrate had to be considerably diluted before the reaction could be slowed down sufficiently to be measurable. Fig. 3 shows the change in the ultraviolet

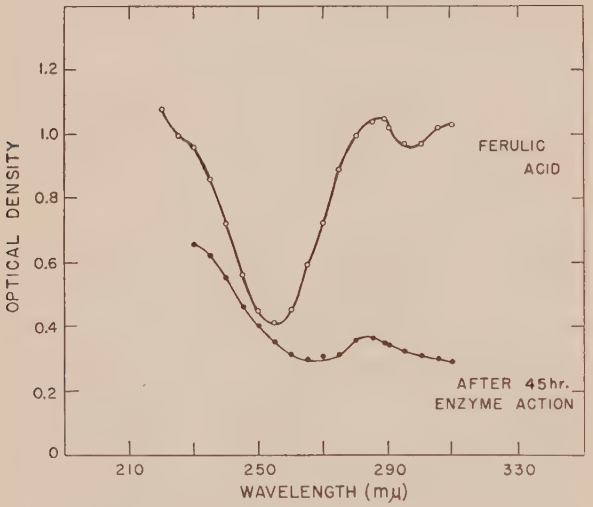


FIG. 3. Change in the ultraviolet absorption spectrum of ferulic acid as a result of enzyme action.

Reaction mixture, 25 ml. 0.00157% ferulic acid containing 10 ml. McIlvaine's buffer at pH 4.80 + 0.5 ml. culture filtrate diluted 1 : 10 with water, incubated at 37° C.

spectrum of ferulic acid after 45 min. of enzyme action, when the reaction was completed. When greater concentrations of substrate were used, the products of the enzyme action precipitated out as fine pinkish-orange particles.

Fig. 4 shows the effect of the enzyme on the ultraviolet spectrum of vanillin. Using the same concentration of substrate as in the ferulic acid experiment, 10 times as much enzyme was necessary to complete the reaction in a reasonable time. It can be seen from Fig. 5 that the rate of change of the vanillin measured at 250 mμ is more rapid than the rate measured at 280 mμ. Since the wave length of 250 mμ is the position of the deepest trough in the vanillin spectrum, changes at this point as a result of enzyme action have no significance in themselves, but are the result of reactions taking place at the two peaks at 230 mμ and 280 mμ. Since the rate of change at 280 mμ, is measurable and is different from the rate at 250 mμ, the disappearance of the trough gives an estimate of the reaction taking place at 230 mμ a wave length at which it is impossible to read with the concentrations used in this experiment. The products of enzyme action also precipitated out of solution

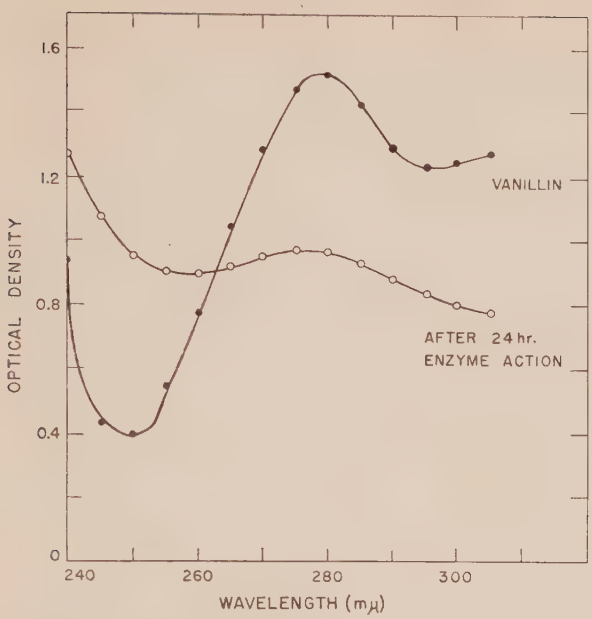


FIG. 4. Change in the ultraviolet absorption spectrum of vanillin as a result of enzyme action.

Reaction mixture, 25 ml. 0.002% vanillin containing 10 ml. McIlvaine's buffer at pH 4.80 + 0.5 ml. culture filtrate incubated at 37° C.

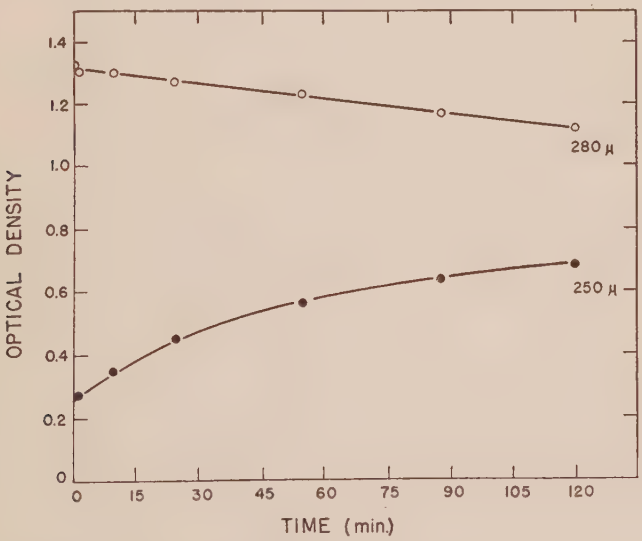


FIG. 5. The rates of enzymatic action on vanillin when optical density was measured at wave lengths of 250 mμ and 280 mμ.

Reaction mixture, 25 ml. 0.0016% vanillin containing 10 ml. McIlvaine's buffer at pH 4.80 + 0.5 ml. culture filtrate incubated at 37° C.

during the reaction, producing a grayish brown substance which gave an ultraviolet spectrum very similar to that of lignin (Fig. 4), and from which vanillin could not be regenerated by nitrobenzene oxidation.

Effect of the Enzyme on Lignin

A rapid darkening of indulin solutions was caused by the enzyme but no visible change could be detected as a result of enzymatic action on "native" aspen or wheat-straw lignin. There was little change in the ultraviolet spectrum of any of the three lignins even after prolonged enzyme action, although there was a slight sharpening of the curves as shown in Fig. 6. Indulin appeared to be brought into solution by the enzyme.

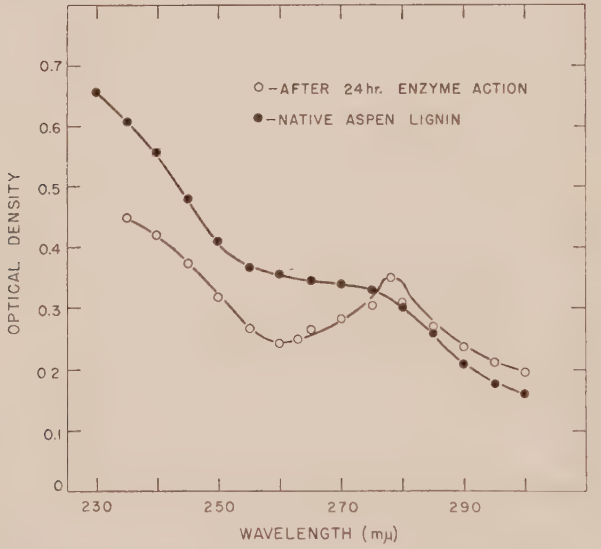


FIG. 6. Effect of oxidase system from PRL 572 on the ultraviolet absorption spectrum of "native" aspen lignin.

Reaction mixture, 25 ml. 0.002% "native" aspen lignin containing 10 ml. McIlvaine's buffer at pH 4.80 + 0.5 ml. culture filtrate, incubated at 37° C.

Uptake of Oxygen as a Result of Enzyme Action

Fig. 7 shows the rate of oxygen absorption as a result of enzyme action on vanillin, ferulic acid, and the three types of lignin. Both vanillin and ferulic acid absorbed oxygen very rapidly at an almost identical rate for the first 40 min. Wheat-straw lignin and indulin took up oxygen more slowly and there was a delay of two hours before the "native" aspen lignin started to absorb oxygen. The differences in rate may be partially accounted for by the fact that the vanillin and ferulic acid were in solution, whereas the lignins were present as fine suspensions. The products of the enzymatic action on

vanillin and ferulic acid precipitated out during the course of the experiment, but the indulin and wheat-straw lignin were partially brought into solution. No visible effect was apparent in the case of the "native" aspen lignin.

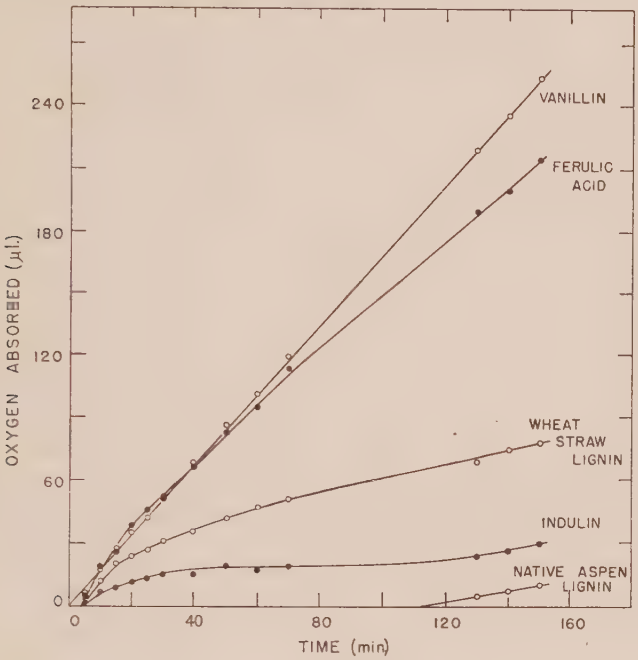


FIG. 7. Absorption of oxygen by lignin, vanillin, and ferulic acid during enzyme action.

Effect of Manganese Sulphate and Magnesium Sulphate on the Action of the Enzyme on Vanillin

Gottlieb and Geller (3) isolated a lignin-oxidizing enzyme system from mushroom spawn. They suspected that two enzymes were present in their preparation, one of which showed its optimum activity at pH values lower than pH 5.2. Since the enzyme produced by PRL 572 also showed its maximum activity in acid solutions it was thought that perhaps it might be identical with this enzyme from mushroom spawn.

Gottlieb and Geller showed that their enzyme was activated by the presence of manganese sulphate. Figs. 8 and 9 show the effect of adding magnesium sulphate and manganese sulphate to the reaction mixture in a concentration of 0.016 *M*, on the action of the PRL 572 enzyme on vanillin. When the change in optical density of the ultraviolet absorption spectrum of the reacting mixture was measured at 250 mμ, the presence of excess manganese sulphate considerably enhanced the rate of reaction, whereas the presence of magnesium sulphate slowed it down. These results are in line with those of Gottlieb and

Geller. However, the change in density at 280 mμ gave completely different results. The presence of additional manganese ions altered the reaction. The peak, instead of being cut off, was enhanced and the final absorption

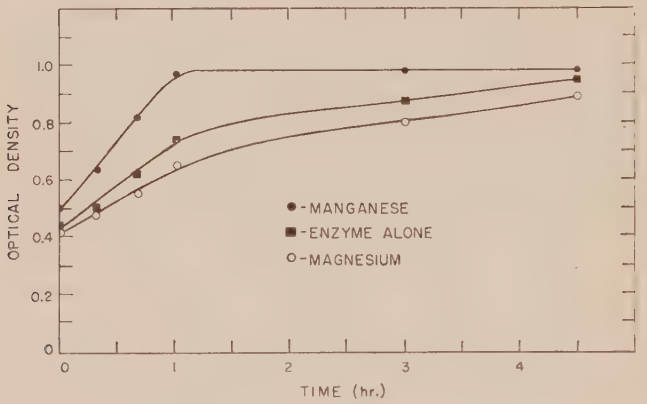


FIG. 8. Effect of additional manganese and magnesium ions on the rate of enzymatic action on vanillin measured at a wave length of 250 mμ.

Reaction mixture, 25 ml. 0.002% vanillin containing 10 ml. McIlvaine's buffer at pH 4.80 and 0.5 ml. culture filtrate incubated at 37° C. Concentrations of manganese sulphate and magnesium sulphate 0.016 *M*.

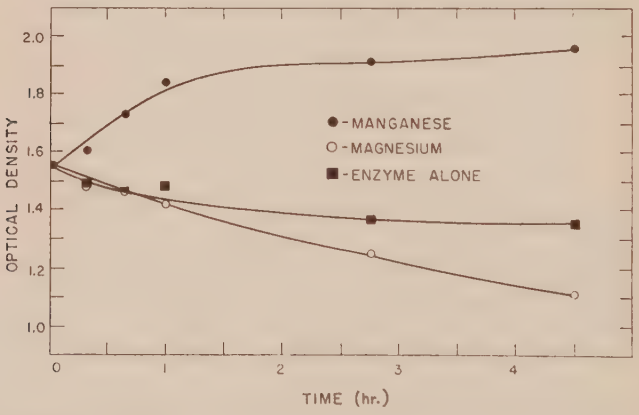


FIG. 9. Effect of additional manganese and magnesium ions on the rate of enzymatic action on vanillin measured at a wave length of 280 mμ.

Reaction mixture, 25 ml. 0.002% vanillin containing 10 ml. McIlvaine's buffer at pH 4.80 and 0.5 ml. culture filtrate incubated at 37° C. Concentrations of manganese sulphate and magnesium sulphate 0.016 *M*.

spectrum after the completion of the reaction is illustrated in Fig. 10. It was also noticed that, in the presence of additional manganese ions, the products of the enzyme action did not precipitate out, as was usual after the action of the enzyme alone or with magnesium sulphate.

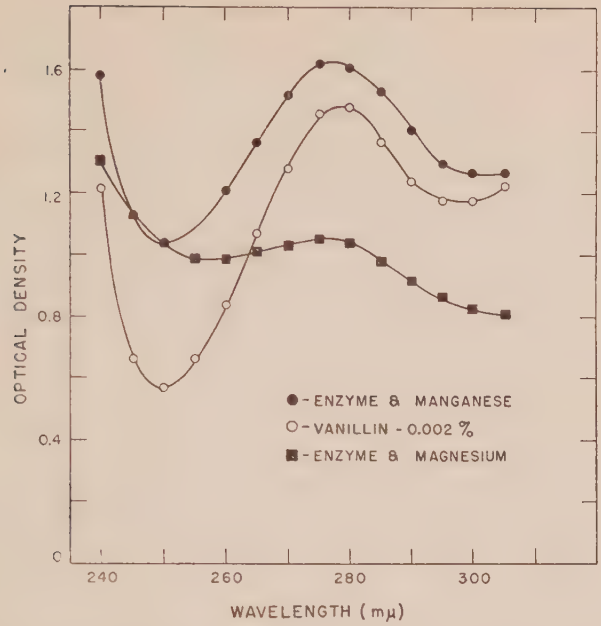


FIG. 10. Effect of enzyme on the ultraviolet absorption spectrum of vanillin after 24 hr. incubation in the presence of additional manganese and magnesium ions.

Reaction mixture, 25 ml. 0.002% vanillin containing 10 ml. McIlvaine's buffer at pH 4.80 and 0.5 ml. culture filtrate incubated at 37° C. Concentrations manganese sulphate and magnesium sulphate 0.016 *M*.

Discussion

This brief preliminary investigation of the oxidase system of *P. versicolor* shows that it is produced abundantly by the organism in submerged culture. As is usual in fermentations for the production of extracellular enzymes, high yields were only obtained as a result of good growth of mycelium. Such growth was obtained when organic nitrogen sources and readily available carbohydrates were used in the culture medium. It is possible that this enzyme is representative of the oxidases which distinguish the so-called white rots from other wood-destroying fungi. It is further possible that this enzyme is similar to the phenol oxidase shown by Law (4) to be produced by white rots and not by brown rots, and which is able to decolorize dyes such as neutral red and gentian violet.

The enzyme system is distinguished from the better known phenol oxidases by its low pH optimum and its stability in acid solution, although it is likely that several enzymes are concerned, each of which may show its maximum activity at different pH values.

The change of color observed when phenols are ozidized enzymatically is often ascribed to *ortho*-quinone formation. Many of the phenols listed in Table I are capable of forming an *ortho*-quinone, but a number of others, such as syringaldehyde, 5-nitro-, and 5-bromovanillin, have the *ortho* position

blocked by groups which are resistant to oxidation. It would seem likely that the color changes produced with such *ortho*-substituted phenols are due to the formation of a *para*-quinone, the carbon atom or atoms *para* to the $-OH$ group being oxidized by the enzyme to a second hydroxyl group. In the case of syringaldehyde and 5-bromovanillin, the aldehyde group could be oxidized in a similar manner to that found in the Dakin reaction.

Assuming that the color changes observed with *ortho*-substituted phenols were due to *para*-quinone formation, it was of interest to determine what side chains could be so oxidized. *l*-Syringylpropene, with an unsaturated linkage in the side chain, was oxidized moderately easily, and, more unexpectedly, β-syringylethanol was also oxidized. If that color change was in fact due to the oxidation of the $-CH_2 . CH_2OH$ group to an $-OH$ group, then the enzyme system under consideration would seem to have very strong oxidizing properties.

It is possible that even in those cases where *ortho*-quinone formation is theoretically possible, as in the case of vanillin, ferulic acid, etc., the enzyme system produces a reaction of the Dakin type with the formation of a *para*-quinone. It also appears that vanillin and ferulic acid are polymerized as well as oxidized. Answers to these speculations can be obtained only by the isolation and identification of the products of the reactions. Such work is beyond the scope of the present paper.

The enzyme system appeared to have a definite, if less spectacular, effect on the three types of lignin investigated. Its action resulted in the uptake of oxygen by the lignins, in an increase in solubility, and in a sharpening of the ultraviolet absorption spectrum as shown in Fig. 6. Lignin, like other substances with a guaiacyl nucleus, may be oxidized to form an *ortho*-quinone. This reaction would account for the darkening of the indulin solutions, but would show little change in the ultraviolet absorption spectrum. The oxidized form appears to be more soluble in water than the unoxidized. This may be due to the additional $-OH$ group, or to a slight depolymerization caused by enzymatic action which would also account for the change in the ultraviolet spectrum.

It may be possible that the role of this enzyme system in nature is to solubilize the lignin and make it susceptible to the action of other enzymes. However, indulin, after being acted upon by the PRL 572-enzyme system, was not made available, as a carbohydrate source, to fungi such as *Aspergillus niger*, *Trichoderma viridi*, or *Penicillium* sp. which were also unable to utilize unchanged indulin.

It is difficult to draw any conclusions from these preliminary experiments, but it appears that the extracellular oxidase system produced by *P. versicolor* resembles the enzyme preparation obtained from mushroom spawn by Gottlieb and Geller in that it is active in acid solution and is affected by the presence of manganese and magnesium ions. However, since isolates of *P. versicolor* have been shown to be able to grow on isolated "native" lignin

as the sole source of carbon, this fungus must possess an enzyme system, capable of decomposing lignin, in which the oxidase described above is possibly a link.

Acknowledgments

The author is indebted to Dr. Nobles for the cultures of *P. versicolor* and to Dr. J. M. Pepper for the gift of the "native" aspen lignin and other phenolic compounds. Grateful acknowledgment is made to Dr. A. C. Neish and Mr. M. Chisholm for the measurements of oxygen absorption, and to Dr. J. E. Stone for his valuable help and advice.

References

1. Dakin, H. D. The oxidation of hydroxy derivatives of benzaldehyde, acetophenone and related substances. Am. Chem. J. 42 : 477-498. 1909.
2. Day, W. C., Pelczar, M. J., and Gottlieb, S. The biological degradation of lignin. I. Utilization of lignin by fungi. Arch. Biochem. 23 : 360-369. 1949.
3. Gottlieb, S. and Geller, J. H. The biological degradation of lignin. II. Isolation and properties of a lignin-oxidizing enzyme. National Military Establishment (U.S.). Symposium on Wood. Washington, June, 1949.
4. Law, K. Phenol oxidases in some wood-rotting fungi. Ann. Botany, n.s. 14 : 69-78. 1950.
5. Ledingham, G. A. and Adams, G. A. Biological decomposition of chemical lignin. II. Studies on the decomposition of calcium lignosulphonate by wood destroying and soil fungi. Can. J. Research, C, 20 : 13-27. 1942.
6. Pelczar, M. J., Gottlieb, S., and Day, W. C. Growth of *Polyporus versicolor* in a medium with lignin as the sole carbon source. Arch. Biochem. 25 : 449-451. 1950.

CYTOGENETICAL RESPONSES OF CEREALS TO 2,4-D

I. A STUDY OF MEIOSIS OF PLANTS TREATED AT VARIOUS STAGES OF GROWTH[1]

By John Unrau[2] and E. N. Larter[3]

Abstract

Different plots of Olli barley, Thatcher common wheat, and Stewart durum wheat were each sprayed with a single treatment of 2,4-D. The spraying was done at three-day intervals from emergence to heading time in order to study the effect of the herbicide when applied to plants at various developmental stages. An ester formulation equivalent to 12 oz. acid in 40 gal. of water per acre was used in all cases. Meiosis was studied in spikes obtained from plants of treated and untreated check plots. This material was fixed and stored in Carnoy's Fluid A. Meiotic irregularities were induced by the treatment in some plants of each of the three cereals at all stages of growth. The percentages of abnormal metaphases and anaphases ranged from 0.0 to 35.3 in barley, 0.0 to 33.3 in durum wheat, and 0.0 to 25.0 in common wheat. Chromosomal aberrations were of various types including bridges, fragmentation, asynapsis, aneuploidy, polyploidy, chain and ring formation, and in many cases extreme stickiness of chromosomes. These aberrations are similar to those induced by X irradiation or other mutagens. It is important to determine whether chromosomal aberrations in treated plants are transmissible and also whether gene mutations have been induced. Cytogenetical studies are under way on progenies of treated plants.

Introduction

During recent years the control of weeds and insect pests has been greatly enhanced by the use of organic chemical compounds. Many field experiments with hormonal herbicides on the effectiveness (for practical weed control) of concentration, form, and time and method of application, have been conducted. Also, basic researches relating to the plant's response, physiologically and morphologically, to various treatments with the different compounds have currently become more prominent.

To date, however, only limited studies have been undertaken to study herbicidally induced irregularities of chromosome behavior or of cell division. This lack of cytological work is perhaps surprising, especially since some morphological effects of these compounds are strikingly similar to those of known mutagens such as X rays. If these compounds should, under certain conditions, alter the hereditary constitution of crop plants, their indiscriminate use could have far-reaching and grave effects, especially in relation to pure seed stocks of presumably stable genetic constitution.

For a review of the possible mode of penetration, translocation, and action of the chemical, the reader is referred to a paper by Blackman (1).

The present studies were undertaken primarily to determine whether treatments of cereals with 2,4-D at different stages of growth would result

[1] *Manuscript received June 14, 1951, and, as revised September 4, 1951.*

Contribution from the Division of Genetics and Plant Breeding, Department of Plant Science, University of Alberta, Edmonton, Alta., with financial assistance from the National Research Council of Canada.

[2] *Professor of Plant Science.*

[3] *Formerly Student Assistant, at present Graduate Assistant in Genetics and Plant Breeding.*

in transmissible cytological and genetical changes. In this, the first of a series of papers, results from cytological studies of meiosis of plants treated with 2,4-D are presented and discussed.

Review of Literature

Doxey (2), Doxey and Rhodes (3), and Nygren (5), have reported that mitoses in root tips of onions and other plants were seriously affected by 2,4-D and related herbicides. Doxey and Rhodes (4) indicated that similar chromosomal disturbances were caused by some of the commonly used organic insecticides. None of these investigators, however, has studied meiosis, nor have they attempted to determine whether transmissible cytogenetic changes were induced. Nygren (5) draws attention to the similarity of cytological effects produced by X rays and 2,4-D treatments and warns that genetic changes may be induced by this herbicide.

Unrau and Corns (7), in a preliminary report of this study, have shown that 2,4-D caused meiotic disturbances in cereal plants treated at different stages of growth.

Materials and Methods

For this study, plots of Olli barley, Stewart durum wheat, and Thatcher common wheat were sprayed with a solution of ester formulation of 2,4-D equivalent to 12 oz. acid per acre. The herbicide was dissolved in water equivalent to field applications of 40 gal. per acre. A single spraying was given each plot and applications were made at three-day intervals from emergence to heading.

For cytological examination, sampling was done when the plants were at microsporogenesis. Plants from untreated check plots were used as controls. Unfortunately the dry weather during the summer of 1950 so hastened development that in some treatments the meta- and anaphases I of meiosis were missed. All material was fixed and stored in Carnoy's Fluid A until used. In a few cases deterioration of the stored material had occurred so that no information could be obtained. The acetocarmine smear method was used for slide preparation. Microphotographs were taken of freshly prepared material at a magnification of approximately 1000 diameters.

Results

The results from cytological studies of meiosis of cereals treated with 2,4-D at different stages and of untreated check material are summarized in Table I.

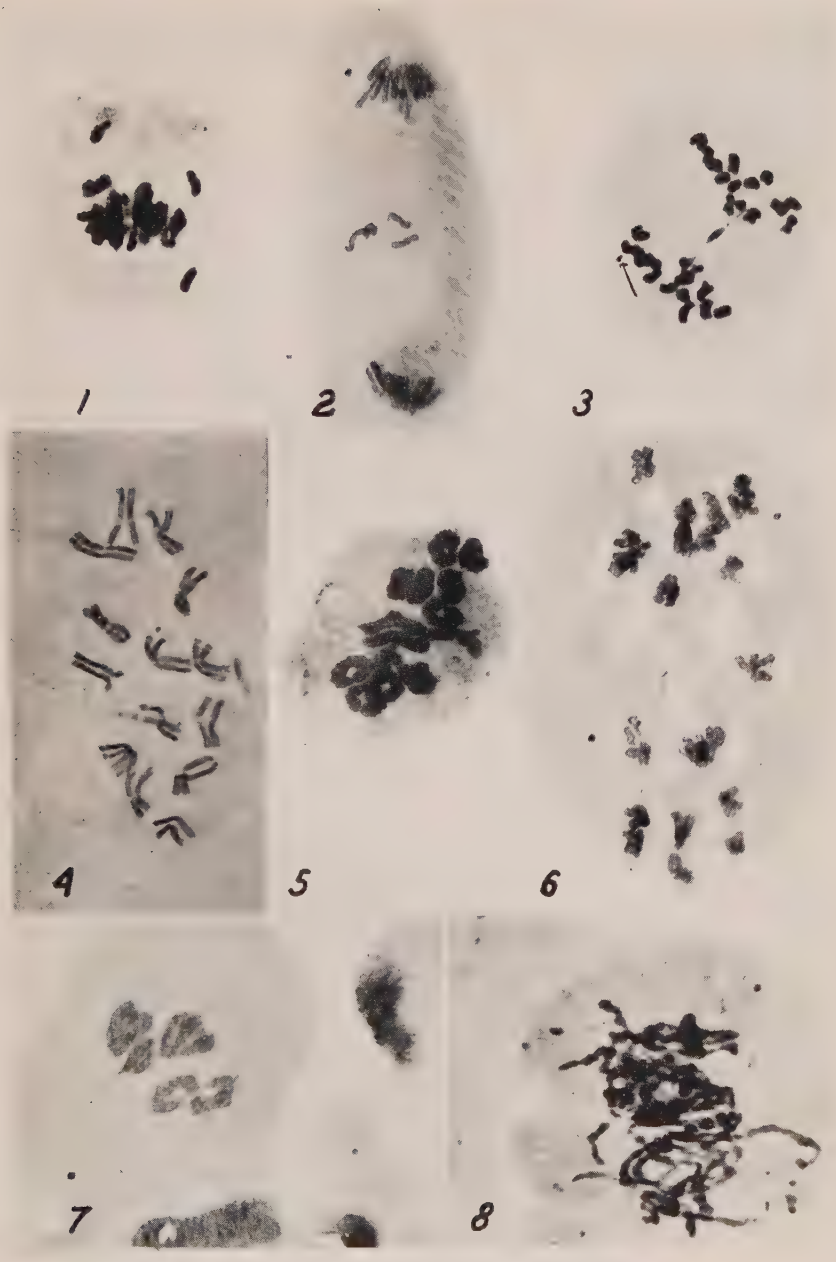
Treatments with 2,4-D caused meiotic irregularities at all stages in all three cereals. All treatments caused marked cytological aberrations in at least some plants. Actually it is surprising that plants having such a high percentage of irregularities (up to 35.3%) were capable of survival.

The variability in percentage of irregular meioses between plants within treatments (0.0 to 35.3%) was likely caused by uneven coverage of the

TABLE I

CYTOLOGICAL EFFECTS OF 2,4-D ON CEREAL CROPS TREATED AT DIFFERENT DATES

Treatment date	No. of plants studied	Number of cells with aberrations						Total abnorm. cells, no.	Total norm. cells, no.	% Abnorm. cells	% Range in treatment
		Univ.	Bridge or fragment	Chains or rings	Aneupl.	Polypl.	Sticky				
Olli barley											
At emergence	6	1	1	1	3	0	2	8	388	2.0	0.0– 6.7
6 days after	3	0	8	0	3	0	1	12	274	4.5	3.6– 7.7
9 days after	4	2	6	0	9	1	8	26	549	4.5	0.6– 7.6
12 days after	7	10	20	1	8	6	16	61	1364	4.5	0.0–35.3
15 days after	5	0	9	0	2	5	2	18	544	3.2	1.0–27.3
18 days after	3	0	5	0	4	0	2	11	212	4.9	1.6– 9.3
Check	5	0	0	0	0	0	0	0	565	0.0	0.0– 0.0
Stewart durum wheat											
3 days after	7	8	24	24	1	2	2	61	358	14.6	0.0–33.0
6 days after	1	0	2	1	0	0	1	4	130	3.0	
9 days after	10	9	14	3	0	0	2	28	526	5.3	0.0–13.6
12 days after	11	38	24	2	0	0	6	70	311	18.4	7.4–33.3
18 days after	5	10	38	19	0	0	3	70	414	14.5	3.6–21.8
21 days after	6	38	25	31	0	0	2	96	794	10.8	2.8–26.3
24 days after	2	3	15	0	0	0	0	18	185	8.9	8.4– 9.5
33 days after	7	23	20	0	0	0	0	43	563	7.1	1.1–16.7
Check	12	0	0	0	0	0	0	0	520	0.0	0.0– 0.0
Thatcher common wheat											
At emergence	6	10	5	11	0	1	10	37	406	8.4	0.0– 9.2
3 days after	8	20	55	10	5	0	0	90	632	12.5	4.8–25.0
6 days after	7	18	20	1	5	1	0	45	493	8.4	5.2–18.2
12 days after	3	3	5	1	0	0	4	13	131	8.8	5.7–17.9
15 days after	6	28	26	0	0	0	5	59	370	13.8	7.2–18.2
21 days after	4	9	7	0	0	0	1	17	151	11.4	6.5–11.9
24 days after	7	14	17	2	0	0	7	40	441	8.3	3.8–20.0
30 days after	5	2	10	0	0	0	12	24	265	8.3	5.5–14.9
Check	10	4	0	0	0	0	0	4	365	1.1	0.0– 1.1



Meiotic irregularities caused by treatments with 2,4-D

FIG. 1. Univalents at metaphase I. From Stewart durum wheat treated nine days after emergence. FIG. 2. Lagging univalents dividing at anaphase II. From Olli barley treated 12 days after emergence. FIG. 3. Bridge and fragment (arrow). From Stewart durum wheat treated three days after emergence. FIG. 4. Tetraploid cell at metaphase. From root tip of Olli barley germinated in 10 p.p.m. solution of 2,4-D. FIG. 5. Tetraploid metaphase I. From Olli barley treated 12 days after emergence. FIG. 6. Aneuploidy in anaphase I. From Olli barley treated nine days after emergence. FIG. 7. Zigzag ring of four at metaphase I. From Olli barley treated 12 days after emergence. FIG. 8. Sticky and corroded chromosomes at metaphase I. From Thatcher wheat treated three days after emergence.

spray. Some plants probably escaped while others received a heavier than normal dose. It is quite conceivable that field spraying likewise would not give absolutely uniform coverage to all plants under all conditions.

The types of aberrations resulting from 2,4-D treatments are exceedingly interesting and significant.

In a large number of affected microsporocytes the aberration consisted of univalent chromosomes (Figs. 1, 2). Functional gametes formed from such cells, could result in monosomics, trisomics, or other aneuploid progeny.

Bridges and fragments were frequently observed in material from all three cereals. Fragmentation probably resulted mostly from anaphase breakage of "sticky" chromosomes or from a process termed "corrosion" by some investigators. In Fig. 8, a cell is illustrated in which extreme breaking up of sticky chromosomes is evident. If this had occurred in preceding divisions, fragments might still be present at meiosis.

Bridges were likewise observed in a number of microsporocytes (Fig. 3). These could possibly result from tardy disjunction or stickiness of individual anaphase chromosomes. On the other hand they could have resulted from joining of ends of nonhomologous chromosomes thus giving dicentric chromosomes.

Polyploidy (Fig. 5) very likely resulted from disturbed chromosome or cell division cycles or both. In a preliminary study, chromosome reduplication without centromere splitting was observed in root tip cells of barley seeds germinated in a 2,4-D solution (Fig. 4). Whether polyploidy at meiosis resulted from chromosome reduplication without anaphasic separation or through failure of cell wall formation at a preceding mitosis cannot be determined.

It is very probable that aneuploidy (Fig. 5) was caused by failure of disjunction of sticky chromosomes or nonsplitting of some centromeres in divisions preceding meiosis.

Chains and rings (ring illustrated in Fig. 7) are likely the result of reciprocal translocations induced in mitoses preceding reduction division.

Stickiness of chromosomes was observed in a large number of the pollen mother cells examined (Fig. 8). In extreme cases, all of the chromatin was present in a continuous undifferentiated clump. This condition of stickiness and also of corrosion of chromosome material might result from changes in the chemical or electrostatic properties of the nucleic acids of the chromosomes. Another interpretation could be that cells so affected are in a state of pycnosis caused by genetic unbalance of cells affected earlier. If this were the case, however, one would not expect such cells to be in the same stage of the division cycle.

Discussion

2,4-D applied to barley and wheat at any stage of growth produced various types of chromosomal aberrations. These results demonstrate, therefore, that there is no developmental stage at which spraying may be done without endangering meiotic stability. Consequently it is not possible to correlate

these results with those of Olson *et al.* (6) who found that yield reductions resulted only when spraying was done at certain specific developmental stages of the plants.

It was surprising to find aberrations in pollen mother cells of plants sprayed at emergence. These aberrations could be caused by either (1) persistent transmission of abnormalities induced immediately on application of the herbicide, or (2) residual persistent activity of the chemical. The latter appears to be the more probable explanation. Had the aberrations been induced on application, one would expect that whole sectors of the plant would be similarly affected; certainly all microsporocytes in a floret. Actually, however, various types of aberrations were found in the anthers of a single floret. This is taken as evidence that the herbicide was still active weeks after application.

The important points still to be determined are: (1) whether the various types of chromosomal aberrations observed at meiosis in treated plants are transmitted, and (2) whether observable genetic changes have been induced by the herbicide.

Certainly one would expect inversions, fragmentation effects, translocations, and aneuploidy to be transmitted, but one should not forget that 2,4-D has now been in general use for a number of years and there are no reports of mutant plants from research workers or grain farmers. It may be that cytologic or genetic changes have occurred but have gone undetected through lack of systematic cytogenetic search. On the other hand, it is possible that seriously affected pollen is rarely effective in fertilization in competition with good pollen, thus more or less preventing aberrations found in microsporocytes from becoming hereditary.

With respect to transmission through the egg, it is possible that seriously affected megaspores do not function at fertilization. This would explain the poor seed set often found in treated plants.

It is not considered possible to draw any definite conclusions as to transmissibility of observed irregularities on the basis of the present results. It is hoped that this question will be clarified by cytological and genetical studies of succeeding generations. These further studies are in progress and the results will be reported in due course.

Acknowledgments

The authors take pleasure in expressing their thanks to Dr. L. P. V. Johnson, Professor of Genetics and Plant Breeding, University of Alberta, who has read and criticized the manuscript.

References

1. Blackman, G. E. The principles of selective toxicity and the action of selective herbicides. Science Progress, 150 : 637-651. 1950.
2. Doxey, D. The effect of isopropyl phenyl carbamate on mitosis in rye (*Secale cereale*) and onion (*Allium cepa.*). Ann. Botany (n.s.), 13 : 329-335. 1949.

3. Doxey, D. and Rhodes, A. The effect of the plant growth regulator, 4-chloro- 2-methylphenoxyacetic acid on mitosis in the onion (*Allium cepa*). Ann. Botany (n.s.), 13 : 105-111. 1949.

4. Doxey, D. and Rhodes, A. The effects of the gamma isomer of benzene hexachloride (hexachlorocyclohexane) on plant growth and mitosis. Ann. Botany (n.s.), 15 : 47-52. 1951.

5. Nygren, Axel. Cytological studies of the effect of 2,4-dichlorophenozyacetic acid, 2-methyl, 4-chlorophenozyacetic acid and 2,4,5-trichlorophenoxyacetic acid on *Allium cepa*. Ann. Roy. Agr. Coll. Sweden, 16 : 723:728. 1949.

6. Olson, P. J., Zalik, S., Breakey, W. J., and Brown, D. A. Sensitivity of wheat and barley at different stages of growth to treatment with 2,4-D. Agron. J. 43 : 77-83. 1951.

7. Unrau, John and Corns, W. G. Cytological and physiological effects of 2,4-D applied to cereal grains at different stages of growth. Research Rept. Seventh Ann. N. Central Weed Conf. p. 279. 1950.

SYNTHESIS AND HYDROLYSIS OF SUCROSE BY WHEAT LEAVES, AS DETERMINED BY THE VACUUM INFILTRATION METHOD[1]

By G. Krotkov[2] and W. Constance G. Bennett[3]

Abstract

Using the technique of vacuum infiltration, either a mixture of glucose plus fructose or a solution of sucrose alone was introduced into detached wheat leaves, and the rates of sucrose synthesis or hydrolysis from these infiltrated sugars were observed. It was found that these rates were not constant during 24 hr., but depended on the time of the day or night when the leaves were cut. Synthesis was high in leaves detached in the forenoon, before sunset, and in the first part of the night. Changes in the rate of hydrolysis were usually mirror images of those of synthesis. It has been concluded that in wheat leaves there exists a diurnal rhythm in their synthetic and hydrolytic potential and that the observed diurnal changes in the rates of sucrose synthesis and hydrolysis represent one of the manifestations of such a rhythm. Sodium fluoride, sodium cyanide, iodoacetate, and dinitrophenol always increased hydrolysis, and usually decreased synthesis. When glucose-1-phosphate, fructose-6-phosphate, and fructose-1, 6-diphosphate were substituted for the corresponding free sugar in a mixture of glucose plus fructose, a decrease in sucrose synthesis was observed. After 24 hr. of starvation the rate of sucrose synthesis declined, and eventually dropped practically to zero at the end of five days. In the same time there was a progressive increase in the rate of hydrolysis.

Introduction

The method of vacuum infiltration (9, 10) appears to be singularly suited for the investigations of the synthesis and hydrolysis of sucrose *in vivo*. Using this technique, solutions containing known amounts of either a mixture of fructose and glucose or sucrose are introduced into plants, and the experimental material is then placed under controlled conditions for several hours. At the end of this time the plants are analyzed, and the amounts of sucrose synthesized from infiltrated glucose and fructose, or the amounts of glucose and fructose produced by hydrolysis from sucrose, are determined. It is also possible to infiltrate into plants, together with sugars, some additional substances, e.g. respiratory poisons, whose effect on sucrose synthesis or hydrolysis it is desirable to ascertain. Moreover, instead of infiltrating a mixture of glucose and fructose, one can introduce some suspected intermediaries, e.g. phosphorylated sugars, in order to observe synthesis of sucrose from them.

In view of all these advantages it was decided to try this method, and the results obtained are presented on the following pages.

[1] *Manuscript received November 20, 1951.*

Contribution from the Department of Biology, Queen's University, Kingston, Ont., with financial help from the National Research Council of Canada and the Science Research Committee of Queen's University.

[2] *Professor of Biology.*

[3] *Holder of a Research Council of Ontario scholarship during part of this investigation. Present address, Division of Botany, Central Experimental Farm, Ottawa, Ont.*

Materials and Methods

Throughout this investigation Marquis wheat has been used.* Plants were grown either in flats or in 5 in. pots in a University greenhouse, no attempt being made to control external conditions. Plants were grown trimmed, to consist solely of the first leaf, and unless stated otherwise they were 12-15 days old when used for the experiments. Physiologically, therefore, leaves were present at the stage of early maturity (5). Unless stated to the contrary, leaf samples were always taken between 9-10 a.m. The technique of vacuum infiltration and a sample of calculations for the determination of the rates of sucrose synthesis and hydrolysis are described elsewhere (9, 10). Duplicate determinations of such rates on comparable leaf material agreed within 4%. Sugars were determined by the Hagedorn and Jensen method, and the sucrose content was calculated from the increase in reducing power after hydrolysis with hydrochloric acid. The general procedure for sugar extraction and subsequent analysis was described earlier (7).

When Newton (14) analyzed wheat leaf extracts for their reducing power after inversion with either invertase or hydrochloric acid, he obtained similar results. On the basis of this he concluded that in wheat leaves sucrose is the only oligosaccharide present. The same conclusion has been reached by Roberts (15). Bidwell (1), using the technique of paper chromatography, found that in attached wheat leaves, even after 16 hr. of illumination, the only oligosaccharide present was sucrose. On the other hand comparable detached leaves after similar illumination contained large amounts of alcohol soluble fructosans in addition to sucrose. The last worker was unable, however, to observe, in darkness, synthesis of fructosans from infiltrated sugars. From this he concluded that fructosan synthesis in detached wheat leaves takes place only in light.

In the present investigation, leaves were always kept in darkness following infiltration with sugars. In view of all the evidence presented above it was assumed that in such leaves sucrose was the only oligosaccharide present. Under these conditions, its determination from the increase in reducing power following inversion with hydrochloric acid appears to be justified.

Previous to the actual experiments, the suitability of the vacuum infiltration method for the proposed studies was explored. It was thought that infiltration of aqueous sugar solutions into intercellular spaces might cut down the oxygen content of the internal atmosphere to such an extent that the transformations of sugars would be depressed (13). Moreover, the rate of sugar intake from the intercellular spaces into cells might be slow enough to become the limiting factor in the observed synthesis or hydrolysis of sucrose. It might also be pointed out that a large proportion of infiltrated sugars is lost in respiration and not in the transformations under study.

In order to determine the effect of nonremoval of infiltrated water from the intercellular spaces, samples of leaves were infiltrated with either distilled

* *The seeds were kindly supplied by F. H. McNeal of the Montana Experimental Station, to whom the authors express their thanks.*

water, 0.1 *M* sucrose or with a 0.2 *M* mixture of glucose and fructose. One-half of each sample was placed directly into a desiccator, the atmosphere of which was saturated with water vapor, and kept there in darkness for three hours at 25° C. At the end of this time the leaves were extracted with 85% ethanol and the rates of sucrose synthesis and hydrolysis were determined.

The second half of each sample was strung on pins and placed for 10 min. in a constant temperature and light chamber at 25° C. in front of an electric fan. Preliminary tests have indicated that such a treatment brought the weight of leaves down to practically the same value which they had before infiltration, and that this drying had no effect on either absolute or relative amounts of various sugars present in the leaves. After such a drying, leaves were placed in a desiccator and treated as those described above. This test was repeated on three consecutive days with the results shown in Table I.

TABLE I

THE EFFECT OF NONREMOVAL OF WATER FROM THE INTERCELLULAR SPACES ON SYNTHESIS AND HYDROLYSIS OF SUCROSE*

	Synthesis (as mgm. sugar/hr./gm. leaf fr. wt.)	Hydrolysis (as mgm. sugar/hr./gm. leaf fr. wt.)	$\frac{\text{Synthesis}}{\text{Hydrolysis}}$ ratio
Leaves nondried	0.68	1.70	0.40
Leaves dried	0.88	1.23	0.71

* *Average of three tests.*

From Table I it is clear that nonremoval of water following infiltration decreases synthesis and increases hydrolysis. In all subsequent work leaf samples after infiltration were always dried as described above.

Disappearance of infiltrated sucrose from the intercellular spaces was observed as follows: A solution of sucrose was infiltrated into leaves and these were placed in a desiccator for one hour. At the end of this time leaves were infiltrated with invertase solution, left in the desiccator for 30 min. longer and then analyzed for their reducing sugars. If at the end of one hour after infiltration with sucrose, this sugar was completely absorbed into the cells, then subsequent infiltration of invertase would have no effect on the reducing sugars content of such leaves. If, on the other hand, some sucrose still remained in the intercellular spaces, then the reducing sugars content of this leaf sample should be increased. No increase was observed. From this it has been concluded that the amounts of sucrose infiltrated into intercellular spaces are completely absorbed into cells at the end of one hour.

The same has been assumed for glucose and fructose, since permeability of cells toward these two sugars is even greater than toward sucrose. The results of this test confirm a similar conclusion reached by Kursanov (12).

To determine the effect of infiltration on respiration, leaf samples were infiltrated with distilled water, sucrose, or a mixture of glucose and fructose,

and the respiration of each sample was determined for two hours. It was found that infiltration with water, sucrose, or glucose plus fructose increased carbon dioxide production by about 5%, 50%, and 100% respectively. Converting the excess of carbon dioxide produced in each case into sugar and comparing this value with the amounts of total sugars infiltrated into leaves, it was found that only about 4% of the infiltrated sucrose and 6% of the glucose and fructose were lost in increased respiration. Under the conditions adopted in the present investigation, not less than 30% and usually 50% of infiltrated sugars are involved in either synthesis or hydrolysis. For this reason the error due to the loss of infiltrated sugars in respiration has been disregarded.

Diurnal and Ontogenetic Changes in the Synthesis and Hydrolysis of Sucrose

The rates of synthesis and hydrolysis of sucrose were determined in leaf samples taken from plants at three hour intervals for 24 consecutive hours. The plants were 8, 15, and 28 days old. The results obtained are presented in Figs. 1-3.

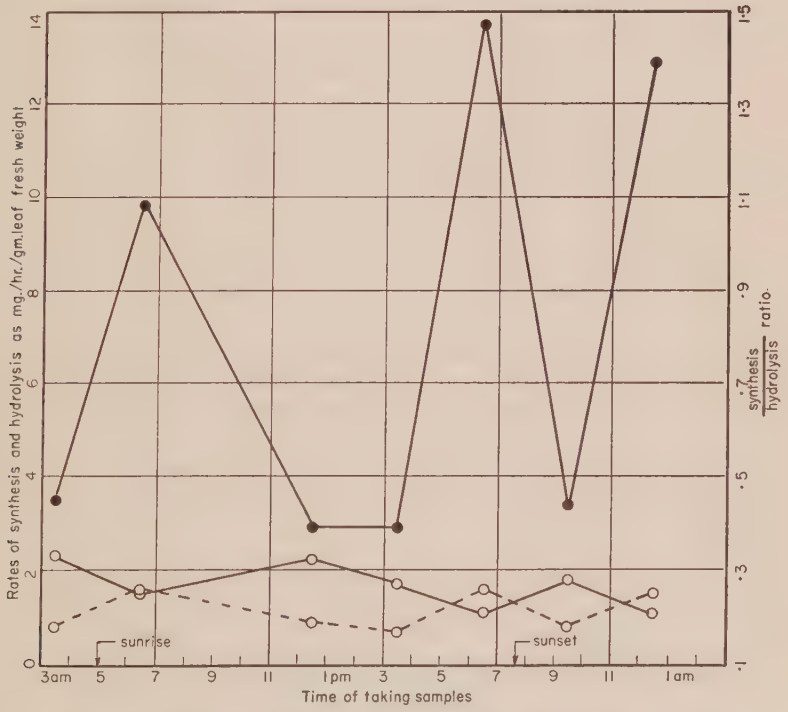


FIG. 1. Diurnal changes in the rates of synthesis and hydrolysis of sucrose in wheat leaves. Juvenile stage. Age eight days. o----------o Synthesis. o————————o Hydrolysis. ●————————● $\frac{\text{Synthesis}}{\text{Hydrolysis}}$ ratio.

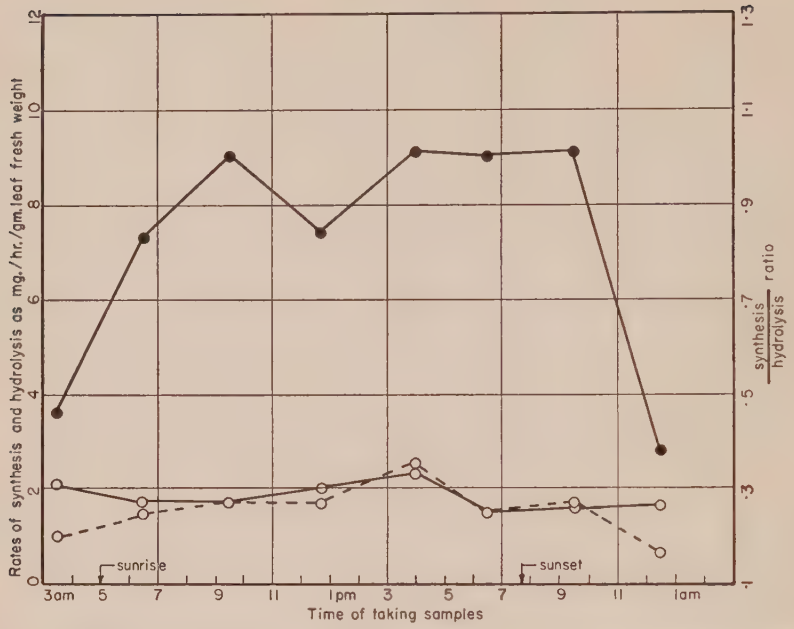


FIG. 2. Diurnal changes in the rates of synthesis and hydrolysis of sucrose in wheat leaves. Mature stage. Age 15 days. O----------O Synthesis. O——————O Hydrolysis. ●——————● $\frac{\text{Synthesis}}{\text{Hydrolysis}}$ ratio.

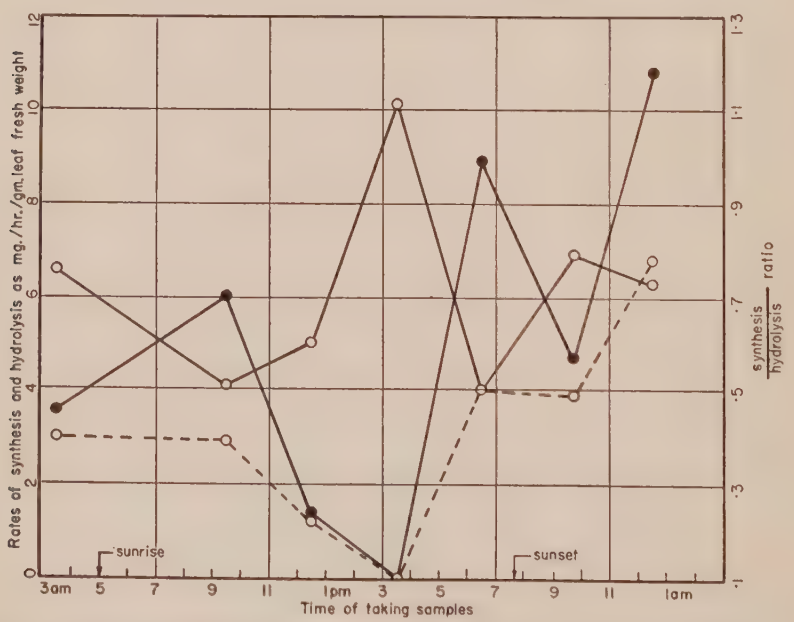


FIG. 3. Diurnal changes in the rates of synthesis and hydrolysis of sucrose in wheat leaves. Senile stage. Age 28 days. O----------O Synthesis. O——————O Hydrolysis. ●——————● $\frac{\text{Synthesis}}{\text{Hydrolysis}}$ ratio.

The rates of synthesis and hydrolysis did not remain constant during 24 hr., but underwent a series of regular changes. Thus the rate of hydrolysis always dropped after sunrise; then it went up, reaching a peak between 12.30 – 4.00 p.m.; declined to a low value before sunset, and then went up again. For both 8- and 28-day-old plants changes in the rates of synthesis appeared to be a mirror image of these for hydrolysis. When hydrolysis went up, synthesis went down, and when hydrolysis declined, synthesis rose. For 15-day-old plants such a relationship was observed only before the sunrise and after sunset, when external conditions were more constant.

From the increase in the fresh weight of leaves following infiltration, one can calculate the absolute amounts of sugars introduced. The analysis of leaves at the end of each experiment gives the actual amount of sugars present, and after correction for respiration, the two should tally. This has been observed in the course of this work only in exceptional cases, and a typical graph showing variations in such a discrepancy during 24 hr. is given in Fig. 4.

The diurnal trend observed is essentially the same no matter whether infiltration was done with sucrose or with a mixture of glucose and fructose. Whenever leaves were taken before sunrise, during the afternoon, or in the

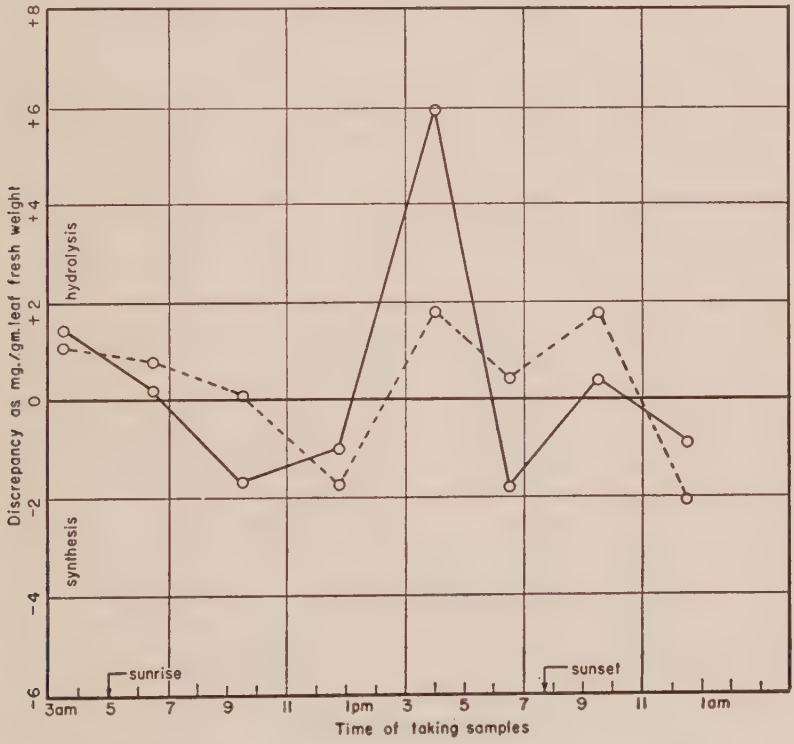


FIG. 4. Diurnal changes in the discrepancy between the analyzed and calculated values for the amounts of sugars in wheat leaves.

○————○ Infiltrated with sucrose. ○- - - - - - - - - - - -○ Infiltrated with glucose plus fructose.

night, more sugars were found than could have been expected from the amounts infiltrated. In leaves taken shortly after sunrise or shortly before sunset, there was a loss of sugars.

Disappearance of infiltrated sugars from the leaves may be explained as due to their transformation into some substances not analyzable as sugars. Appearance of sugars may be due to their release from some unknown source. Those parts of the graph shown in Fig. 4, which are above the zero line, represent an excess of hydrolysis above synthesis, and those below, the reverse. Between 6-10 a.m. both synthesis and hydrolysis appear to be more nearly balanced than in any other time of the day. It is for this reason that samples used in this work were usually taken at this time of the day.

When one compares the graphs showing diurnal changes in the synthesis and hydrolysis of sucrose as given in Figs. 1-3 with those showing the discrepancy between the amounts of sugars infiltrated into leaves and those found by analysis as given in Fig. 4, and finally with the graphs showing diurnal changes in the synthesis and hydrolysis of complex carbohydrates as reported elsewhere (8), one is struck by the similarity in all these graphs. In all of them the highest and the lowest rates of synthesis and hydrolysis occur at about the same time. The easiest explanation of such a synchronization would be to postulate the existence of a diurnal rhythm in the general synthetic and hydrolytic potential of leaves. On this assumption synthesis and hydrolysis of either sucrose, of complex carbohydrates, and possibly a number of other substances are just different examples of the same synthetic or hydrolytic potential.

The observed ontogenetic trends in the synthesis and hydrolysis of sucrose are summarized in Table II.

TABLE II

A SUMMARY OF CHANGES IN THE SYNTHESIS AND HYDROLYSIS OF SUCROSE DURING ONTOGENY

Age of plants, days	Synthesis		Hydrolysis		$\frac{\text{Synthesis}}{\text{Hydrolysis}}$ ratio	Average
	Rate*	Average	Rate*	Average		
8	0.68–1.60	1.12	1.09– 2.31	1.68	0.35–1.47	0.79
15	0.64–2.50	1.53	1.50– 2.34	1.83	0.38–1.08	0.84
28	0 –6.79	3.11	4.02–10.15	6.19	0 –1.18	0.59

* *Mgm. sugar per hr. per gm. leaf fresh weight.*

During leaf ontogeny the rates of sucrose synthesis and hydrolysis both increased. Since the rate of hydrolysis increased more rapidly, the $\frac{\text{synthesis}}{\text{hydrolysis}}$ ratio dropped with the advancing age of a leaf. The amplitude of diurnal variations in synthesis and hydrolysis was smaller in younger leaves than it

was in the older. A similar effect of age on the amplitude of diurnal variations in the sugar content of mangold leaves was reported by Davis, Daish, and Sawyer (3).

Effects of Respiratory Poisons

Leaf samples were infiltrated either with the usual solutions of sugars or with those containing in addition one of the following respiratory poisons: 0.02 *M* sodium fluoride, 0.01 *M* sodium cyanide neutralized, 0.005 *M* iodoacetate, and a 0.45% dinitrophenol. Leaves used as experimental material with the first two poisons were picked as usual between 9 and 10 a.m. and those with the last two at 1 p.m. Experiments were repeated on three consecutive days, and the results are given in Table III.

TABLE III

EFFECT OF RESPIRATORY POISONS ON SYNTHESIS AND HYDROLYSIS OF SUCROSE

	Synthesis (mgm. sugar/hr./gm. leaf fresh weight)			Hydrolysis (mgm. sugar/hr./gm. leaf fresh weight)			$\frac{\text{Synthesis}}{\text{Hydrolysis}}$ ratio		
	Expt. 1	Expt. 2	Expt. 3	Expt. 1	Expt. 2	Expt. 3	Expt. 1	Expt. 2	Expt. 3
Control	1.67	1.72	0.50	0.66	0.96	1.85	2.53	0.92	0.27
+ Sodium fluoride	1.20	1.06	1.24	—	1.90	2.90	—	0.56	0.43
+ Cyanide	0.65	0.48	0.67	2.04	1.62	2.29	0.31	0.29	0.29
Control	2.20	1.96	2.62	2.14	1.57	2.57	1.04	1.25	1.02
+ Iodo-acetate	1.16	0.83	0.16	2.69	1.77	3.38	0.43	0.46	0.04
+ Dinitro-phenol	1.21	1.83	0.00	2.77	3.81	2.97	0.44	0.48	0

An examination of the six control samples reveals that they varied a great deal in their rates of synthesis, hydrolysis, and in the ratios between the two. This indicates that leaf samples used with various poisons were not very close physiologically. In spite of this it may be seen from Table III that in every case there was an increase in hydrolysis under the action of every poison tried. In 10 cases out of 12 there was also a depression of synthesis. In Experiment 3 there was an increase in synthesis under the action of sodium fluoride and sodium cyanide, but this experimental material was characterized initially by an unusually low $\frac{\text{synthesis}}{\text{hydrolysis}}$ ratio.

Synthesis of Sucrose from Phosphorylated Sugars

Since both phosphorylated fructose (6, 11) and glucose (4) have been proposed as possible intermediaries in the synthesis of sucrose, it was decided to test these sugars. In order to conserve the amounts of phosphorylated

sugars necessary for such a test, the total concentration of infiltrated sugars was cut down from the usual 0.2 to 0.1 *M*.

The sugars tested were glucose-1-phosphate, fructose-1, 6-diphosphate, and fructose-6-phosphate. In every case the phosphorylated sugar was used as its potassium salt. To its solution there was added an equimolar amount of either fructose or glucose, and its pH was adjusted to 5.4 which in preliminary tests was found to be that of the wheat leaf cell sap. The results of this test are given in Table IV.

TABLE IV

SYNTHESIS OF SUCROSE FROM PHOSPHORYLATED SUGARS

	Synthesis (mgm. sugar/hr./gm. leaf fresh weight)		
	Expt. 1	Expt. 2	Expt. 3
Control (glucose + fructose)	1.17	1.17	1.17
Glucose-1-phosphate + fructose	0.365	0	0
Fructose-1, 6-diphosphate + glucose	0.079	0	0
Control	0.924	0.924	0.924
Fructose-6-phosphate + glucose	—	0	0

None of the phosphorylated sugars tested brought about an increase in the synthesis of sucrose. In some cases there was a decrease, and in others a complete suppression. Preliminary tests have shown that in wheat leaves sucrose synthesis proceeds equally well from either a mixture of glucose plus fructose, or from glucose or fructose infiltrated singly. This suggests an active glucose $\rightleftharpoons$ fructose interconversion system. On the basis of this one would expect at least some sucrose synthesis from the other component of the infiltrated mixture, even if none of the phosphorylated sugars tested are the actual intermediaries. A complete suppression is quite unexpected.

Work in progress has indicated that under the conditions adopted in the present investigations, about 50% of the glucose-1-phosphate infiltrated into leaves disappears from intercellular spaces. This means that at least glucose-1-phosphate is used by wheat leaves. It might be utilized, however, for some other purpose than sucrose synthesis and its utilization somehow brings about a decrease in other sugars, which otherwise might be used for sucrose synthesis. This supposition is supported by the observation that leaf samples infiltrated with phosphorylated sugars always had a lower sucrose content than those infiltrated with distilled water.

Synthesis and Hydrolysis of Sucrose During Starvation

A large number of leaves were cut at midday when their initial sugar content was high. They were placed in beakers containing distilled water, and kept for five days in a desiccator in darkness at 23° C. with a stream of air passing

over them. The rates of sucrose synthesis and hydrolysis were determined daily on samples of these leaves. This experiment was repeated three times, and typical data obtained are presented in Fig. 5.

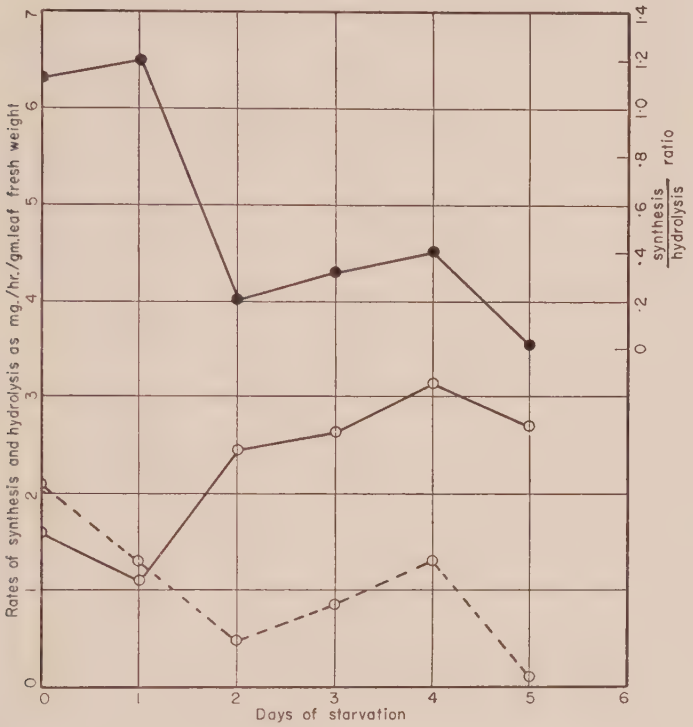


FIG. 5. Changes in the rates of synthesis and hydrolysis of sucrose during starvation. Mature stage. Age 12 days. o----------o Synthesis. o——————o Hydrolysis. ●——————● $\frac{\text{Synthesis}}{\text{Hydrolysis}}$ ratio.

After 24 hr. of darkness the rate of sucrose hydrolysis went up and remained high for the rest of starvation. The rate of synthesis declined for two days, and after a temporary rise between the third and fourth day declined further. The observation of a rise in the rate of sucrose synthesis in the middle of starvation has received a support from an entirely different source. Using the technique of paper chromatography Bidwell (1) reported for wheat leaves a complete disappearance of sucrose after two days of starvation, and its temporary reappearance on the third and fourth day.

This increase in the rate of sucrose hydrolysis during starvation is of considerable interest. According to Blackman (2) a climacteric rise in the carbon dioxide production by stored apple fruits is due to an increase in the hydrolytic processes of their tissues. A similar climacteric rise in the carbon dioxide respired has been observed by many workers in a number of starved plant organs, including wheat leaves (7). The data given in Fig. 5 provide the

actual experimental evidence that, during starvation, at least the rates of sucrose hydrolysis are increased. Moreover, as is evident from Fig. 6, with the progress of starvation more sugars were found in leaves by analysis than

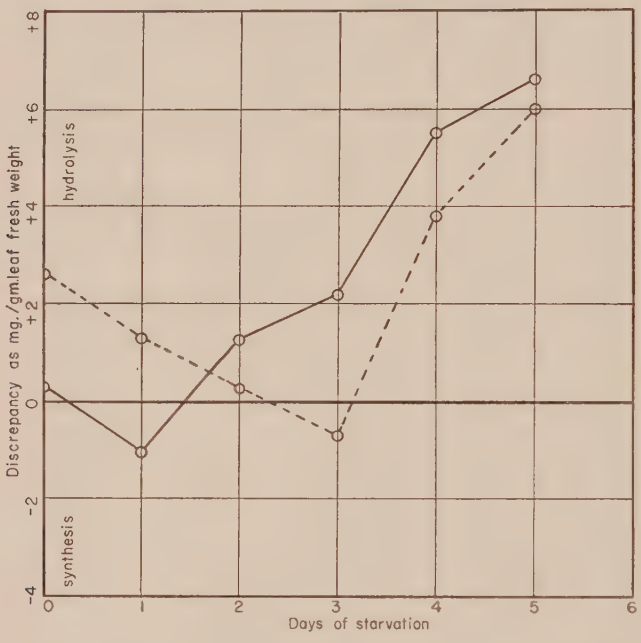


FIG. 6. Changes in the discrepancy between the analyzed and calculated amounts of sugars in starved wheat leaves.
O———O Infiltrated with sucrose. O- - - - - - - - - - - -O Infiltrated with glucose plus fructose.

could be expected from the infiltration data. In starvation, therefore, just as was observed during the normal diurnal changes, an increase in hydrolysis of sucrose is accompanied by an increase in hydrolysis of some unknown substances yielding sugars. This observation lends a further support to the conclusion reached earlier in this paper, that synthesis and hydrolysis of sucrose is closely linked to the general synthetic and hydrolytic potential existing in leaves.

Summary

1. Wheat leaves were vacuum infiltrated with either a mixture of glucose plus fructose or sucrose in solution, and left for several hours in darkness under standard conditions. At the end of this time they were analyzed and the amounts of sucrose synthesized or hydrolyzed from infiltrated sugars were determined.

2. Diurnal variations were observed in the rates of sucrose synthesis and hydrolysis. Synthesis was high in the forenoon, before sunset and shortly after. An inverse relationship was usually observed between the rates of synthesis and hydrolysis.

3. Respiratory poisons like sodium fluoride, sodium cyanide, iodoacetate, and dinitrophenol increased hydrolysis and usually depressed synthesis.

4. Introduction of glucose-1-phosphate, fructose-6-phosphate, and fructose-1,6-diphosphate depressed the sucrose synthesis.

5. In detached leaves after 24 hr. of starvation the rates of sucrose hydrolysis went up and remained high until the disintegration of tissues set in. The rate of synthesis declined for the first two days, and after a temporary rise between the third and fourth day declined further.

6. It is suggested that a diurnal rhythm in general synthetic and hydrolytic potential exists in leaves. Variations in the synthesis and hydrolysis of sucrose, of complex carbohydrates, and possibly of other substances could be considered as different examples of such a rhythm.

References

1. Bidwell, R. G. S. Carbohydrate metabolism in plants. M.A. Thesis, Queen's University. 1951.
2. Blackman, F. F. and Parija, P. Analytical studies in plant respiration. I. The respiration of a population of senescent ripening apples. Proc. Roy. Soc. (London), B, 103 : 412-445. 1928.
3. Davis, W. A., Daish, A. J., and Sawyer, C. C. Studies of the formation and translocation of carbohydrates in plants. I. The carbohydrates of the mangold leaf. J. Agr. Sci. 7 : 255-326. 1916.
4. Doudoroff, M. Kaplan, N., and Hassid, W. Z. Phosphorolysis and synthesis of sucrose with a bacterial preparation. J. Biol Chem. 148 : 67-75. 1943.
5. Duff, G. H. and Forward, D. F. Physiological and biochemical studies in plant metabolism. I. The respiration of the seedling wheat leaf in starvation and ontogeny. Can. J. Research, C, 27 : 125-145. 1949.
6. Hartt, Constance E. The synthesis of sucrose in the sugar plant. Parts I-IV. Hawaiian Planters' Record, 47. 1943.
7. Krotkov, G. Carbohydrate and respiratory metabolism in the isolated starving leaf of wheat. Plant Physiol. 14 : 203-226. 1939.
8. Krotkov, G. Diurnal changes in the carbohydrates of wheat leaves. Can. J. Research, C, 21 : 26-40. 1943.
9. Krotkov, G. Vacuum infiltration as a method for determining enzymic activity in vivo. Science, 105 (2723) : 318-320. 1947.
10. Kursanov, A. L. The use of the vacuum infiltration method for the determination of the synthetic and hydrolytic actions of invertase in living plant tissues. Biokhimiya, 1 : 269-294. 1936.
11. Kursanov, A. L. Participation of phosphatase in the synthesis of sucrose. Biokhimiya, 4 : 229-240. 1939.
12. Kursanov, A. L. and Kriukova. On the rapidity of penetration of sugars introduced in infiltration into the cells. Biokhimiya, 2 : 674-686. 1937.
13. McReady, R. M. and Hassid, W. Z. Transformation of sugars in excised barley shoots, Plant Physiol. 16 : 599-610. 1941.
14. Newton, R. The nature and practical measurement of frost resistance in winter wheat. Univ. Alberta Coll. Agr. Research, Bull. 1. 1924.
15. Roberts, D. W. A. Qualitative and quantitative determination of the wheat leaf carbohydrates. Can. J. Research, C, 28 : 754-779. 1950.

A STUDY OF MEIOSIS IN A HAPLOID OF *TRITICUM VULGARE* VILL. AND ITS PROGENIES[1]

By R. C. McGinnis[2] and John Unrau[3]

Abstract

Meiosis was studied in the haploid of *Triticum vulgare* and in its second and third generation selfed progenies derived from pollination of the haploid with normal pollen. In the haploid, 57.6% of the metaphase cells showed one to three bivalents. This is assumed to be evidence of a degree of homology between chromosomes of the haploid set. Random distribution of univalents to the poles was apparently restricted by bivalent and secondary associations. Cells with 42 chromosomes were observed in a few instances. In one case the complement was made up of 21 bivalents. A high frequency of trivalents and quadrivalents was observed in the second and third generations. Presumably crossing over between partially homologous chromosomes, occurring in the haploid, resulted in reciprocal translocations which were transmitted to succeeding generations. Plants with chromosomal deficiency or duplication, observed in the second and third generations, probably originated from union of unbalanced gametes in first generation plants heterozygous for translocations or deficient or duplicated for one or more chromosomes.

Introduction

A study of chromosome behavior at meiosis in haploids is useful in establishing the phylogeny, the basic chromosome number, and the relationships or homoeologies among chromosomes in a species. Such a study is particularly valuable in polyploid species like common wheat. While common wheat is usually considered to be an allohexaploid, some investigators believe that the three genomes present in this species originated from a common ancestor. If this hypothesis is correct there should still be considerable homology between chromosomes of different genomes. Such homology, if sufficiently extensive would be revealed by bivalent formation in haploid plants.

In this paper the results of cytological investigations of meiosis in a haploid of *Triticum vulgare* Vill.,* and its progenies will be presented and discussed.

Review of Literature

No effort will be made in this paper to give a comprehensive review of the general problem of haploidy. For the most recent review the reader is referred to a paper by Smith (14). Reference will be made here and later to

[1] *Manuscript received in original form June 14, 1951, and, as revised, October 15, 1951.*

Contribution from the Division of Genetics and Plant Breeding, Department of Plant Science, University of Alberta, Edmonton, Alta., with financial assistance from the National Research Council of Canada. This paper is based on a thesis submitted by R. C. McGinnis in partial fulfilment of the requirements for the degree of Master of Science.

[2] *Cytologist, Dominion Laboratory of Cereal Breeding, Winnipeg, Man.*

[3] *Professor of Plant Science.*

* *While Triticum aestivum L. has priority, the authors prefer to use Triticum vulgare Vill., because the latter is more truly descriptive and is also most frequently used by wheat investigators.*

those publications dealing specifically with common wheat haploids or with certain phenomena observed in this study.

Random chromosome distribution to the poles with little or no chromosome pairing has been observed in common wheat haploids by a number of investigators (1, 2, 4, 15, 8). Some investigators, however, have found pairing to occur (1, 3, 6, 7, 9, 10, 14, 16). These workers do not agree in their definitions of chromosome pairing nor in the interpretations of its significance.

Smith (14), Levan (9), and Yamasaki (16) believed that true homology existed if an observable chiasma was present in a bivalent.

Secondary pairing has been observed by a number of workers (14, 3, 15), and by these has been interpreted as evidence of relic chromosome similarity. Others (1, 6), believe that secondary pairing has no relationship to true or relic homology but is merely the result of chance sticking together of prophase chromosomes.

Sears (13), on reviewing his own work and that of earlier workers, concluded that true pairing of haploid chromosomes and the formation of whole chromosome-deficient and duplicated restitution gametes resulted in progenies with reciprocal translocations, and with losses or additions of entire chromosomes.

Materials and Methods

In July, 1949, a haploid of *Triticum vulgare* was observed in an F_3 line of the intervarietal cross Lemhi × (Bunyip × Dicklow). The plant was not noticed until flowering time when small stature and extreme spreading of the glumes drew attention to it. Three spindly tillers were present, of which two were in flower and the third was at sporogenesis. This spike was removed and fixed for cytological studies. The remaining two spikes, as well as two that developed on tillers brought on by unusually heavy rains, were pollinated with Lemhi pollen. Eight seeds were produced. These were sown in the greenhouse in September of 1949. Since each plant had only one tiller, no cytological studies of this generation were attempted. Seven plants grew to maturity and all but one produced seed. The F_2 and F_3* progenies were grown in the greenhouse in February and June of 1950, respectively. Only a few of these plants failed to survive, and all the rest were examined cytologically at microsporogenesis.

Ten seeds from 18 of the haploid's sister plants were also sown in the greenhouse and the chromosomal constitution determined in pollen mother cells. From the information obtained, the chromosomal constitution of the haploid's parent could be inferred.

All cytological material was fixed in 6 : 3 : 1 Carnoy's fluid A, and the acetocarmine smear method was used for slide preparation. Chromosome counts were made at a magnification of 600 diameters and photomicrographs were taken at a magnification of 720 or 900 diameters.

* *Actually these are backcross generations, but for the purposes of this discussion they will be designated as F_2 and F_3 generations.*

Experimental Results

Meiosis in the Haploid

Distribution of Univalent Chromosomes

In general the chromosomes were distributed throughout the entire cell at metaphase I (Fig. 1, Plate I). There was no real evidence of the formation of a true achromatic figure, consequently metaphase I and anaphase I could not be clearly distinguished.

A total of 274 pollen mother cells were counted in which the univalents had migrated to the poles (Figs. 2 and 3, Plate I). The anaphase I distributions are presented in Table I.

Cells in which distribution of the univalents was nearly equal occurred most frequently. Actually, the distribution values in such cells, 11-10, 12-9, 14-7, 15-6, and 17-4, were close to a random distribution. Some of the other distributions, however, deviated very markedly from the expected so that for the entire study a highly significant χ^2 value was obtained. It is quite apparent, therefore, that distribution of chromosomes to the poles was not random for all classes. It is interesting that a 20-1 distribution was observed in two of the cells studied (Fig. 3, Plant I). Sears (11), postulated that since such a distribution would produce a chromosome-deficient gamete, it could provide the mechanism by which a monosomic plant might originate.

Paired Chromosomes

As shown in Table II, a large number of cells with paired chromosomes were observed. Homologous pairing was assumed if a chiasma was actually observed (Fig. 4, Plate I), or if the tapered end appearance of a disjoining pair indicated the previous existence of a chiasma. So-called secondary pairing was also frequently noted, consisting merely of a loose end-to-end association of univalent chromosomes with no sign of a chiasma.

Of a total of 378 pollen mother cells in which the chromosomes were counted at metaphase I, the greatest proportion (31.0% and 30.4%) had a constitution of 19′ 1″, and 17′ 2″, respectively. A constitution of 15′ 3″ was observed in 6.1% of the cells, but no cell was observed that had more than three pairs. These frequencies are quite different from those reported by Yamasaki (16), but quite similar to those of Krishnaswamy (7).

Pairing in the haploid could have resulted from one of two causes. Had the parent of the haploid been heterozygous for a number of reciprocal translocations, bivalent formation in the haploid could be expected. On the other hand, pairing would also occur if there were homology between various chromosomes in the haploid. The first hypothesis can be ruled out since no ring or chain associations were found in the progenies of the haploid sister plants. All 18 sister progenies proved to be completely normal cytologically. The pairing observed must, therefore, be taken as evidence of true homology between parts of certain of the haploid chromosomes resulting in crossing over leading to reciprocal translocations. If the presence of bivalents is a measure of the number of reciprocal translocations that have taken place, it can be

TABLE I

DISTRIBUTION OF UNIVALENTS

	Distribution										
	20-1	19-2	18-3	17-4	16-5	15-6	14-7	13-8	12-9	11-10	Total
No. of cells	2	2	7	2	15	8	31	19	90	98	274
% of total	0.7	0.7	2.6	0.7	5.5	2.9	11.3	6.9	32.9	35.8	100%
Normal (%) distribution	0.002	0.02	0.1	0.6	1.9	5.2	11.1	19.4	28.0	33.6	100%
χ^2	245.000**	23.120**	62.500**	0.017	6.821**	1.017	—	8.054**	0.858	0.144	347.531**

** *Highly significant.*

TABLE II

CHROMOSOME ASSOCIATIONS

	Association										
	21′	19′1″	17′2″	15′3″	21″	42′	21 dyads	Chain of			Total
								3	4	5	
No. of cells	93	117	115	23	1	7	5	11	5	1	378
% of total	24.6	31.0	30.4	6.1	0.3	1.8	1.3	2.9	1.3	0.3	100

assumed that up to three such exchanges have occurred in some cells. It is not suspected that this homology was between chromosomes of the same genome, but rather between chromosomes of different genomes.

While mostly open bivalents were observed, a closed pair with a chiasma on both sides of the centromere was observed in three cells (Fig. 5, Plate I). This has been observed in only one of the other haploids that have been studied. It must be interpreted as crossing over on both sides of the centromere resulting in chromatids having considerable portions interchanged. It also indicates homology of considerably greater portions of certain chromosomes than is usually assumed to exist (11).

Secondary pairing, while frequently observed, is not likely an indication of true homology. Possibly, as Bleier (1) has suggested, stickiness of the chromosomes from prophase on could account for its occurrence.

As mentioned previously, the random distribution of the chromosomes at anaphase I was obviously restricted. Bivalent formation and secondary associations were likely responsible for disturbing the distributions. Regular disjoining of bivalents at anaphase I would result in more than normal of the near equal distributions while associations remaining united and going to the same pole, would result in more than normal numbers of the unequal types. It is, therefore, concluded that distribution was random when 21′ were present, but that when bivalents or associations were present, distribution was random for only those chromosomes not involved.

Chains

Associations or chains of three to five univalents were observed in 4.5% of the cells (Fig. 6, Plate I). Apparently a continuation of secondary pairing was responsible for this type of chain formation. The chromosomes associated in chains would likely go to the same pole, thereby further affecting the randomness of chromosome distribution.

Cells with Reduplicated Chromosomes

One cell was observed that had 21″ at metaphase I (Fig. 1, Plate II). So far as could be determined, such a phenomenon has not been observed in any of the haploids that have been studied. Pairing was normal and very likely normal gametes would result from such a cell. Seven cells were observed that had 42 mostly univalent chromosomes (Fig. 2, Plate II). It is believed that the first type of reduplicated cell could have arisen from failure of cell wall formation during the first mitotic division of a sporogenous cell. When

---

Microsporogenesis in the haploid.

FIG. 1. Metaphase I. Distribution of the 21 unpaired chromosomes throughout the entire cell.

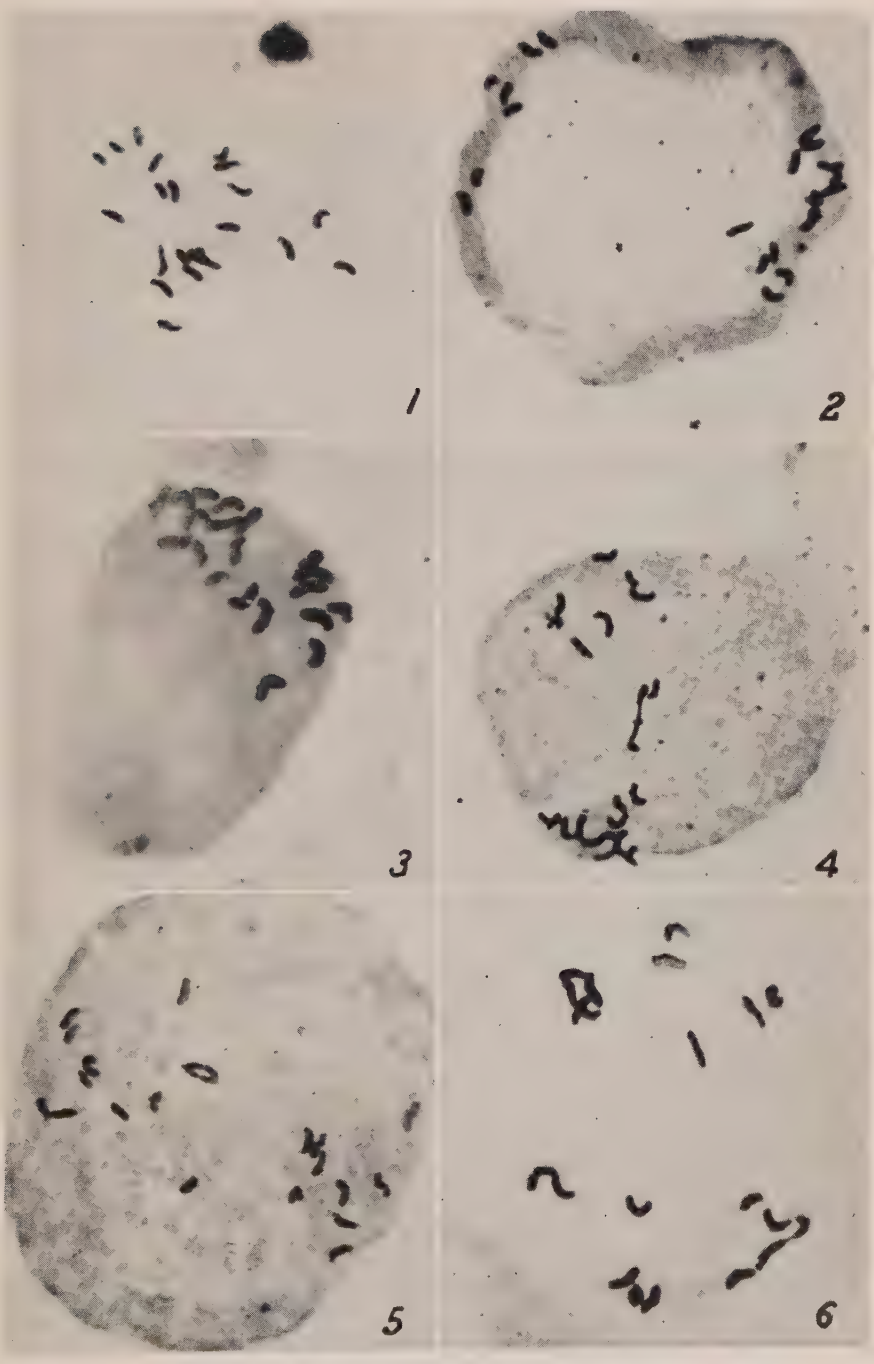
FIGS. 2 and 3. Anaphase distribution; 14-7 and 20-1, respectively.

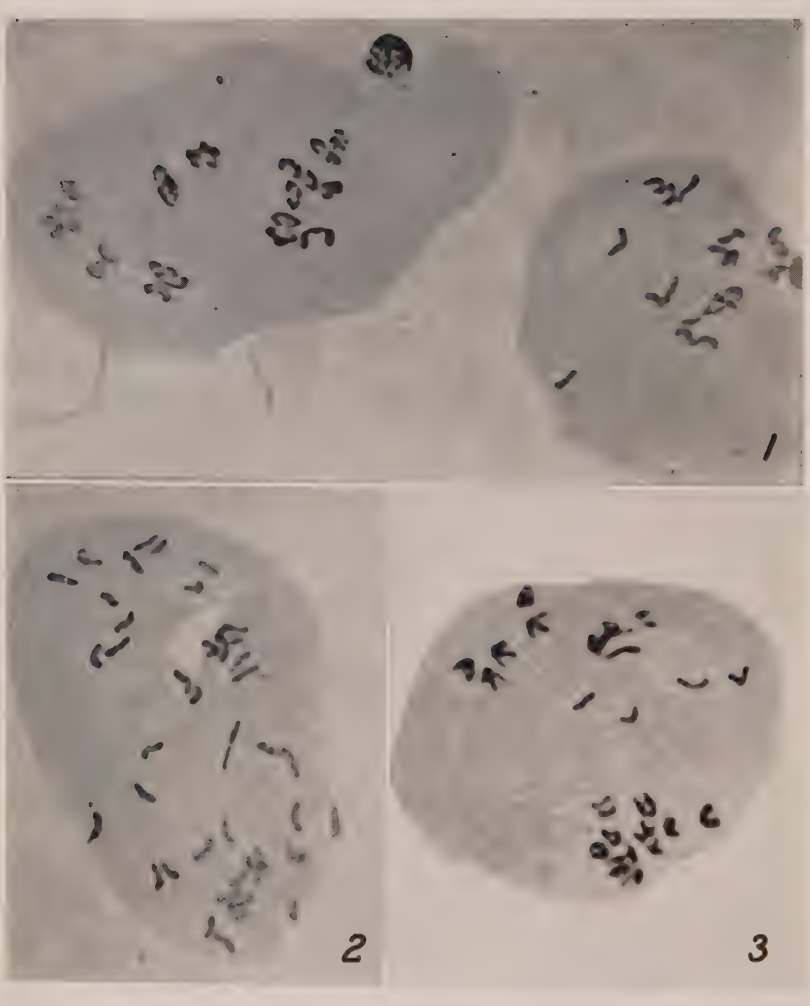
FIG. 4. 19′1″. Open bivalent with strong chiasma.

FIG. 5. 19′1″. Closed bivalent.

FIG. 6. Chain associations.

Magnification: Figs. 3 and 6 at 900 ×; all others at 720 ×.


1
2
3
4
5
6



Microsporogenesis in the haploid.

FIG. 1. Cell with 21 normal pairs; a second cell with 21 univalents.

FIG. 2. Cell with 42 mostly univalent chromosomes.

FIG. 3. Monads and dyads.

All figures at a magnification of 720 ×.

the nuclei so formed prepared for the second mitotic division, a common spindle was formed and the resulting daughter cells (microsporocytes) would have 42 chromosomes each. Meiosis would then proceed normally. In the second case, cells with 42 univalents could have arisen by cell wall failure in the second mitotic division (immediately preceding microsporocyte formation). Since the nuclear membranes in these binucleate cells would remain intact until diakinesis, there would be no opportunity for the 42 chromosomes to pair

Only five cells contained dyads, which were probably formed from a first-division split of the univalents, remaining joined only at the centromere. In Fig. 3, Plate II, it can be seen that some of the univalents have undergone complete splitting and the monads and dyads are being randomly distributed to the poles. It is probable that the dyads would undergo a second division but the gametes thus formed would be deficient for several whole chromosomes and would largely be nonfunctional.

Cytological Data from the F_2 and F_3 Generations

Since no cytological data are available from the F_1 plants, all information on the transmission of aberrations had to be obtained from the analyses of the F_2 and F_3 populations. The chromosomal constitutions of the F_2 and F_3 generations which were cytologically examined, are presented in Table III.

TABLE III

Chromosomal constitutions of F_2 and F_3 generations

F_2 chromosome constitution	Total F_2 plants analyzed	F_3 chromosome constitution	Total F_3 plants analyzed
21″	26	21″	108
21″ or 19″C″ ″	9	21″	24
		21″ or 19″C″ ″	8
		21″ or 19″C″ ″ or 19″C′ ″1′	1
		21″	6
		20″1′	2
21″ or 19″C′ ″1′	3	20″1′ or 19″C′ ″	1
		21″ or 19″C″ ″ or 19″C′ ″ 1′	3
		18″C″ ″1′	1
		21″	3
21″ or 19″C′ ″1′ or 18″2C′ ″	1	21″ or 19″R″ ″	2
		21″ or 17″R″ ″C′ ″1′ or 19″ C″ ″	1
19″C″ ″ or 19″C′ ″1′	1	19″C′ ″1′ + frag.	1
		19″C″ ″1′ + frag.	1
		19″C″ ″	1
18″C″ ″2′	1	19″C′ ″1′	1
		20″2′ + iso.	1
		17″C″ ″C′ ″1′ or 19″C″ ″	1
19″C′ ″ or 17″20′ ″1′	1	—	0

TABLE III—*Concluded*

CHROMOSOMAL CONSTITUTIONS OF F_2 AND F_3 GENERATIONS—Concluded

F_2 chromosome constitution	Total F_2 plants analyzed	F_3 chromosome constitution	Total F_3 plants analyzed
16″C″ ″C′ ″3′	1	19″C′ ″1′	3
		18″C″ ″2′	1
		17″C′ ″4′	1
		16″3C′ ″3′	1
20″1′	4	21″	6
		20″1′	9
		19″2′	1
20″1′ or 19″3′ or 18″C″ ″1′	1	20″1′ or 19″C′ ″ or 19″3′	1
		19″2′	2
19″3′	1	21″	1
		20″1′	1
		19″2′	1
19″2′	2	20″1′	3
		19″1′	3
		19″2′	1
17″C′ ″3′	1	20″1′ or 19″C′ ″	1
		20″1′	2
		19″3′	1
15″C′ ″2′2 + iso.		—	0
21″1′ or 19″R″ ″1′	2	21″1′ or 20″C′ ″	1
		20″1′ or 19″C′ ″	2
		19″4′ or 18″C″ ″2′	1
		18″C″ ″1′ or 17″C″ ″3′ or 20″1′	2
		18″C′ ″2′	1
		21″	1
21″2′ or 20″C″ ″ or 20″4′	1	21″	3
		21″2′ or 20″R″ ″	1
		20″R″ ″C′ ″ or 18″2r″ ″C′ ″	1
		19″C″ ″1′ or 18″C″ ″C′ ″ or 15″C″ ″2C′ ″3′	1
		19″C″ ″ or 19″C′ ″1′	1
19″c′ ″3′ or 18″2C′ ″2′	1	—	0
21″1′ or 20″C′ ″ or 18″C″ ″C′ ″	3	21″1′ or 20″C′ ″	7
		19″C″ ″1′	2
		21″ + frag.	1
		21″	1
Total	60	Total	231

Of the 60 F_2 plants examined, 26 or 43% had a normal chromosomal complement of 21″. Of 231 F_3 plants examined, 153 or 66% proved to have a normal constitution. Consequently, these data indicate that plants with chromosomal aberrations became progressively fewer with succeeding generations.

As would be expected, all normal F_2 plants had normal progeny. Aberrant plants, however, transmitted aberrations to some of their offspring. Furthermore, the aberrations varied markedly in different sister progenies, being in some cases more extreme than in the parent. Apparently the degree of parental deviation from normality determined the relative frequency of normal and abnormal progeny.

The most common aberrations were chains of three or four chromosomes, rings of four, and univalents. It is assumed that these aberrations were largely the result of reciprocal translocations that occurred in the haploid. Moreover, the high frequency of F_2 and F_3 plants containing up to three associations of three or four chromosomes indicated that three or more chromosomes with interchanged portions were commonly present in functional female restitution gametes of the haploid. Disjunction from translocation configurations in the F_1 likely caused the formation of deficient and duplicated gametes which would result in plants having chains and also unpaired chromosomes, especially if the interchanged portion were small. A diagrammatic representation of the probable origin of aberrant gametes from one reciprocal translocation, is presented in Diagram 1. Random union of any two of such

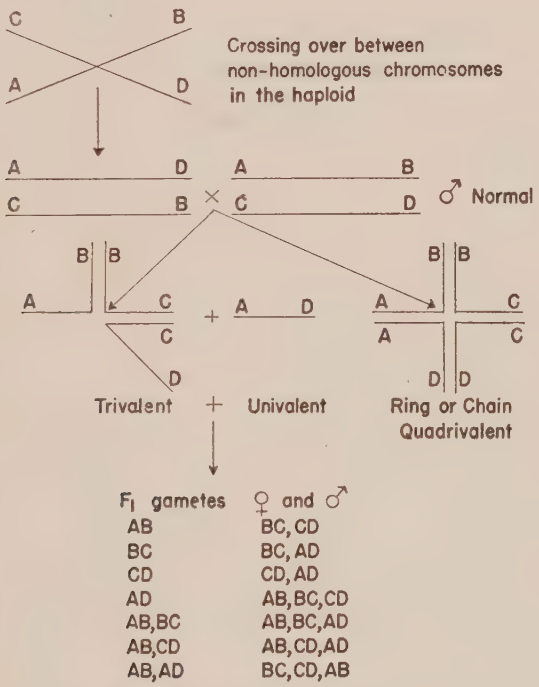


Diagram 1. Diagrammatic representation of the probable origin of aberrations from the haploid.

gametes gives numerous possibilities for various types of chromosomal aberrations. If as many as three reciprocal translocations were involved, the resulting progeny could have all the cytological abnormalities observed in this study (Figs. 1 to 5, Plate III). It is probable that most male gametes would be normal in chromosome number, but occasionally ones with an aneuploid number might be functional. Female gametes, on the other hand, would be expected to transmit aberrations with a fairly high frequency.

The total number of chromosomes in different fertile plants ranged from 39 to 47 (Table III). It is surprising that fertility and vigor were high even in some of the most aberrant plants. The plant with 39 chromosomes appeared quite normal phenotypically. This is highly interesting since Sears (11) found that nullisomics were usually quite weak and sterile. Probably the 39-chromosome plants had certain interchanged chromosomes so that there was actually no complete absence of essential genetic material.

The meiotic behavior of one F_3 plant should be especially noted (Fig. 6, Plate III). At metaphase I, it proved to be, like nulli-3 described by Sears (12), highly asynaptic. It is possible that this plant was nullisomic for chromosome three, and the origin of such a plant can be explained from the union of certain gametes, each deficient for the same chromosome, Diagram 1.

Acknowledgment

The authors wish to express thanks and appreciation to Dr. L. P. V. Johnson, Professor of Genetics and Plant Breeding, for reading and criticizing the manuscript.

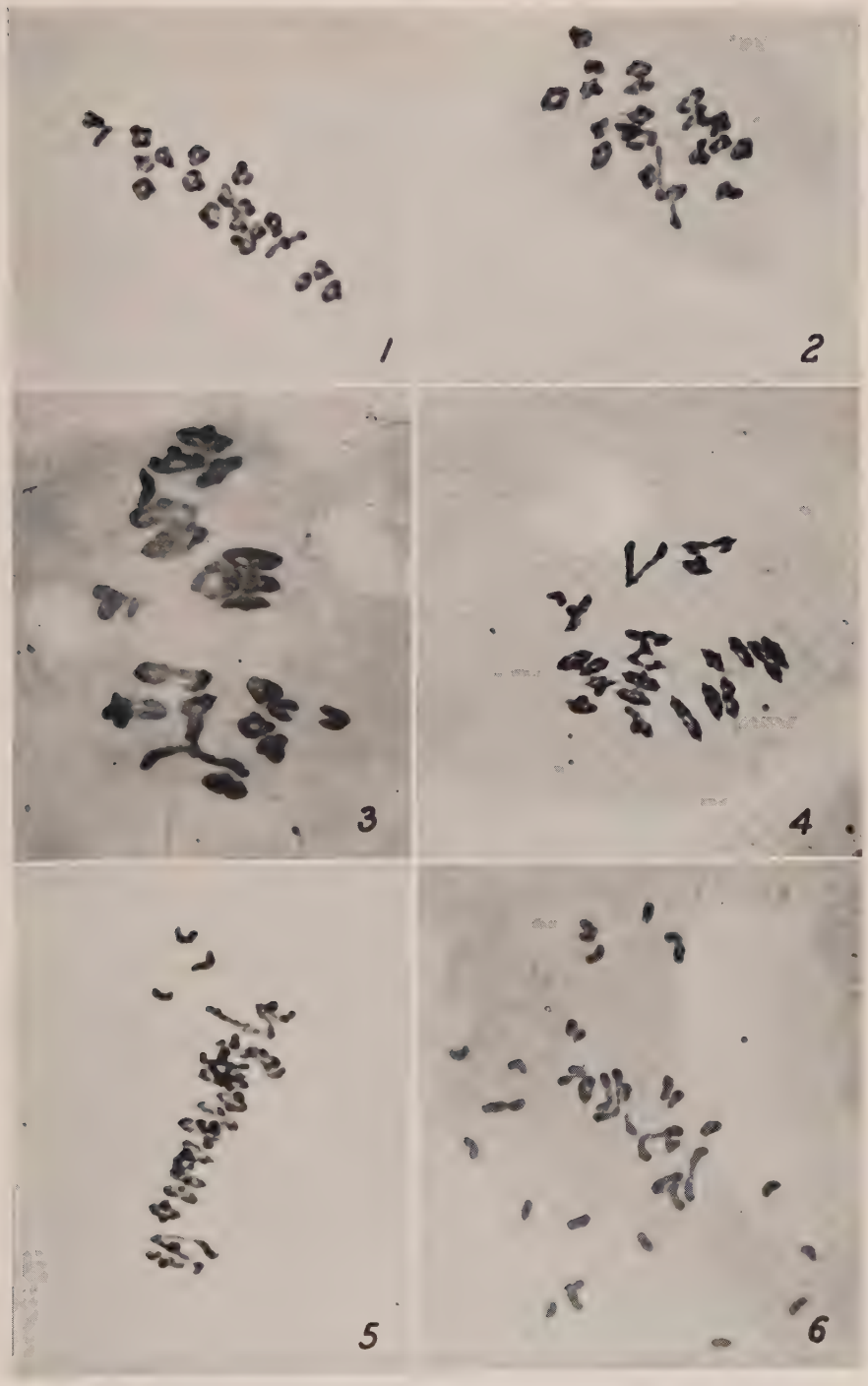
References

1. Bleier, H. Die Meiosis von Haploidiplonten. Genetica, 15 : 129-177. 1933.
2. Davis, B. M. and Kulkarni, C. G. The cytology and genetics of a haploid sport from *Oenothera franciscana*. Genetics, 15 : 55-80. 1930.
3. Flovik, K. Cytological studies of Arctic Grasses. Hereditas, 24 : 265-376. 1938.
4. Gaines, E. F. and Aase, H. C. A haploid wheat plant. Am. J. Botany, 13 : 373-385. 1926.
5. Ivanov, M. A. Experimental production of haploids in *Nicotiana rustica* L. (and a discussion of haploidy in flowering plants). Genetica, 20 : 295-397. 1938.
6. Kostoff, D. The problem of haploidy. (Cytogenetic studies on *Nicotiana haploids* and their bearings to some other cytogenetic problems). Bibliographia Genet. 13 : 1-148. 1928.
7. Krishnaswamy, N. Cytological studies in a haploid plant of *Triticum vulgare*. Hereditas, 25 : 77-86. 1939.

Microsporocytes of F_2 and F_3 plants.

Fig. 1. F_3 cell with 19″1′.
Fig. 2. F_2 cell with 19″C″ ″.
Fig. 3. F_2 cell with 19″R″ ″1′.
Fig. 4. F_3 cell with 19″C′ ″1′.
Fig. 5. F_3 cell with 16″3C′ ″1′.
Fig. 6. F_3 cell. Highly asynaptic.

Magnification: Fig. 3 at 900 ×; all others at 720 ×.


1
2
3
4
5
6

8. LESLEY, M. M. and FROST, H. B. Two extreme "small" *Matthiola* plants: a haploid with one and a diploid with additional chromosome fragments. Am. Naturalist, 42 : 22-33. 1928.
9. LEVAN, A. Studies on the meiotic mechanism of haploid rye. Hereditas, 28 : 177-211. 1942.
10. NORDENSKIOLD, H. Studies of a haploid rye plant. Hereditas, 25 : 204-210. 1939.
11. SEARS, E. R. Cytogenetic studies with polyploid species of wheat. I. Chromosomal aberrations in the progeny of a haploid of *Triticum vulgare*. Genetics, 24 : 509-523. 1939.
12. SEARS, E. R. Cytogenetic studies with polyploid species of wheat. II. Additional chromosomal aberrations in *Triticum vulgare*. Genetics, 29 : 232-246. 1944.
13. SEARS, E. R. The cytology and genetics of the wheats and their relatives. Advances in Genetics, 2 : 239-270. 1949.
14. SMITH, L. Haploidy in einkorn. J. Agr. Research, 73 : 291-301. 1946.
15. TOMETORP, G. Cytological studies on haploid *Hordeum distichum*. Hereditas, 25 : 241-254. 1939.
16. YAMASAKI, Y. Some observations on the microsporogenesis of the haploid plant of *Triticum vulgare* host. Japan. J. Botany, 8 : 151-153. 1936.

PHYSIOLOGICAL AND BIOCHEMICAL STUDIES IN PLANT METABOLISM

V. THE EFFECT OF CHANGES IN SOIL WATER CONTENT ON THE PHYSIOLOGICAL HETEROGENEITY IN THE FIRST LEAF OF WHEAT[1]

By D. W. A. Roberts[2]

Abstract

The respiratory rate, soluble nitrogen content, protein nitrogen content, water content, reducing sugar content, and sucrose content of each of the four quarters of the first leaf of Khapli Emmer wheat have been determined for plants grown with different soil water supplies. Under dry conditions the first leaf of wheat contains a higher concentration of nitrogenous substances than it does under moist conditions. The water content of the first leaf of wheat grown in dry soil or very wet soil is lower than it is for leaves grown in moderately moist soil. The significance of these two observations is discussed. Further support has been obtained for the view that the concentration of none of the substances determined is solely responsible for the respiratory rates and gradients observed in the leaves. The role of leaf anatomy in controlling and producing the observed respiratory gradients is discussed.

Introduction

In the preceding paper of this series (13) the diurnal changes in the gradients in respiratory activity, water content, protein and soluble nitrogen content, reducing substance, and sucrose content were followed. It was concluded that none of the changes in the distribution of any one of the above-mentioned constituents alone was, by itself, responsible for the observed changes in respiratory rate. The suggestion was offered that anatomical features of the leaf might be an important factor in the observed physiological heterogeneity. It is well known that appreciable changes in anatomy can be induced by changed water relations. Preliminary experiments with Khapli wheat showed that changed soil water relations caused changes in the respiratory rate and respiratory gradient. In view of these preliminary findings an examination of the effect of changed soil water relations on the respiration gradient, water content gradient, soluble and protein nitrogen gradients, reducing substance gradient, and sucrose gradient was made.

Methods

The plants were grown under the same conditions that were used in the preceding parts of this study (1, 12) except with regard to water supply to the plants.

In the earlier studies the cans of wheat plants were supplied with 60 ml. of tap water or nutrient solution daily. In the present set of experiments daily water supplies of 15 ml., 30 ml., 45 ml., 60 ml., 75 ml., 100 ml., and 120 ml.

[1] *Manuscript received October 11, 1951.*

A contribution from the Department of Botany, University of Toronto, Toronto, Ont. An abridgment of part of a thesis presented in 1948 to the University of Toronto in partial fulfilment of the requirements of the degree of Doctor of Philosophy.

Plant Physiologist, Laboratory of Botany and Plant Pathology, Lethbridge, Alta.

were used. In the case of plants supplied with 15 ml. of water daily, all those leaves which were full grown at 11 days were used. For plants grown with 30 ml. of water per day leaves in the three $\frac{1}{2}$-cm. size classes, including the modal class, and the two classes on each side of the mode, were used. In the experiments on the effect of soil moisture on the respiratory gradients in the leaves, the samples were taken after one or two hours of darkness (unless stated otherwise in the text or tables) on the 13th day. Samples for the determination of the gradients in nitrogenous substances, reducing substances, and sucrose were harvested at the times indicated in the tables.

The respiration record was determined by the Barcroft method at 22.2° C. and the "stabilized respiration rates" (13) were deduced from this record. All respiration rates quoted are "stabilized" values except as indicated in the text or tables. Soluble and protein nitrogen were determined by the Kjeldahl method, using mercury as a catalyst, after the leaf nitrogen had been divided into the two fractions as described earlier (11). Sucrose and reducing substances were determined as recommended in an earlier publication (10).

Changes in Morphology

The most highly modified leaves are those grown with a supply of only 15 ml. of water per day. These plants are backward, their leaves are frequently inrolled along the margins and usually are wilted in the tip sections during the periods of illumination. The leaves are also darker and bluer green in the plants with 15 ml. of water daily than in the other plants. It is unlikely that the plants could be grown to maturity under these conditions, while there is no reason to suppose that any of the plants given the other daily water supplies could not be grown to maturity. Plants supplied with 30 ml. of water daily are also backward, and their leaves are somewhat smaller than normal, and slightly darker green. The average values for the lengths and widths of leaves of the plants grown with different supplies of soil water are

TABLE I

THE EFFECTS OF SOIL WATER SUPPLY ON THE SIZE OF WHEAT LEAVES

Size of leaf	Water supplied daily, ml.						
	15	30	45	60	75	100	125
Length, cm.	9.9	11.5	12.7	12.6	12.6	12.2	11.7
Width, mm.	3.1	4.3	4.6	4.9	4.7	—	4.3

given in Table I. It is interesting to note that the largest leaves occur on the plants whose leaves have the greatest percentage of water when mature, i.e. the leaves of the plants supplied with 60 ml. of water daily.

Changes in Respiration Rate

The changes in the respiration rate of wheat leaf quarters with changing soil water supply are given in Fig. 1 which shows the average values of the determinations on three different populations of the respiration rate at each soil water level. From this graph it is quite clear that the rates of oxygen

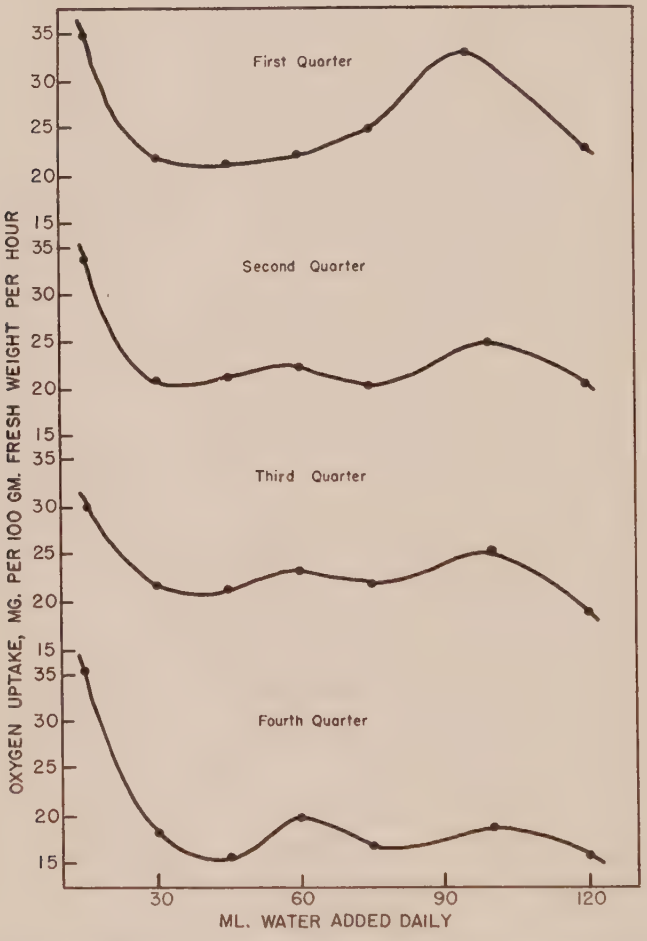


FIG. 1. The changes in the respiration rates of the four quarters of 13-day-old wheat leaves induced by different daily water supplies. The leaves were harvested after one to two hours of darkness.

uptake calculated on the basis of fresh weight are very high in all quarters when the water supply is very low. In the tip quarter, there is also a high rate of oxygen uptake in the leaves of plants grown with 100 ml. of water supplied daily. There was considerable scatter in the values of the respiration rate for this quarter with high water supply. It has been found to be generally true that the higher the respiration rate, the greater the scatter of the values

obtained. The significance of the other humps on the curves is doubtful in view of the relatively few data.

In Fig. 2 the data are presented to show the respiratory gradients with different soil water supplies. Excluding the fourth quarter there is a strong gradient in 15 ml. daily leaves from the tip to the third quarter. However,

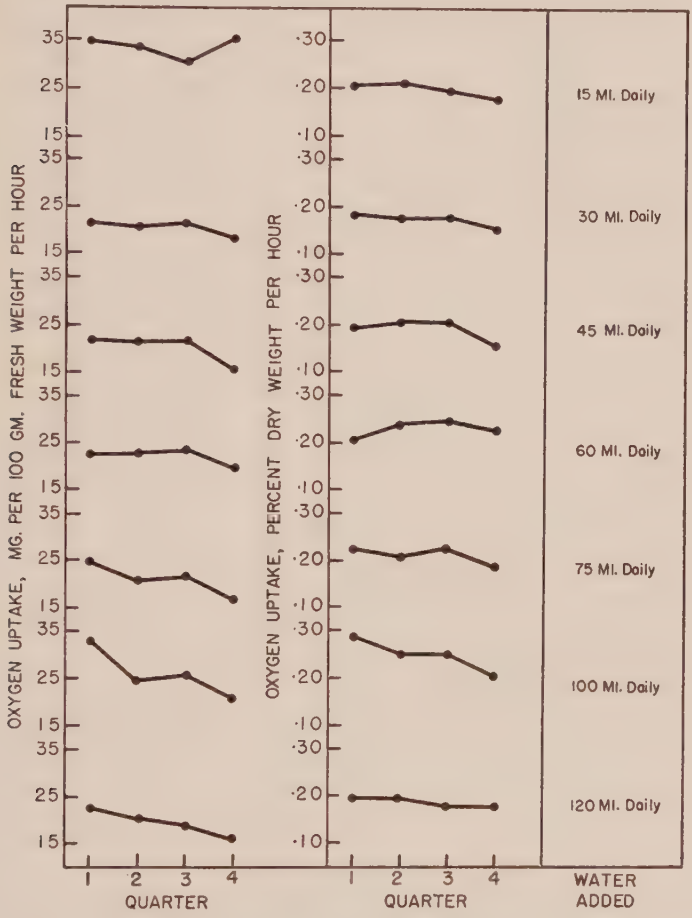


FIG. 2. The gradients in respiration rate of wheat leaves from plants supplied with various amounts of water daily and harvested after one to two hours of darkness on the 13th day after seeding.

the rate for the fourth quarter is higher than that of the third quarter. In the leaves of plants supplied 30 ml., 45 ml., and 60 ml. of water daily the gradients are very similar. The rates of oxygen uptake for the upper three quarters are nearly equal, while the rates for the ligule quarters are decidedly lower. In the leaves of plants supplied with 120 ml. daily there is a uniform gradient from the tip to the base of the leaf with the highest rates of oxygen uptake in the tip quarter and the lowest in the basal quarter. A similar state

of affairs is found in 75 ml. and 100 ml. daily leaves except that the rate of respiration of the third quarter is higher than that of the second quarter.

Fig. 2 also shows that the very high values obtained for fresh weight respiration of 15 ml. daily plants are largely due to the relatively great amount of solid matter in these leaves and not to greatly increased activity on the part of the protoplasm. Examining the figure further it is found that the greatest dry weight respiration rates occur in plants supplied with 60 ml. to 100 ml. of water per day. Low values of dry weight respiration rate are found in leaves from plants given 30 or 120 ml. of water per day.

The respiration gradients calculated on the basis of dry weight are also shown in Fig. 2. In the leaves of plants supplied with little water (15 ml. or 30 ml. daily), and those with a large quantity of water (100 ml. and 120 ml daily), there is a gradient in the rate of oxygen uptake calculated on the basis of dry weight from tip to base, with the highest rates in the tip quarter. In the leaves of plants supplied with 75 ml. of water per day, the respiration rates for the upper three quarters are similar while the rate for the basal quarter is lower. In the leaves of the 45 and 60 ml. daily plants, the rates in the tip and basal quarter are lower than those in the two middle quarters.

Now that the effects of changing soil moisture have been described, the question arises as to whether it is possible to take plants grown to the 13-day stage with a water supply of 60 ml. per day, and by withholding water or supplying excessive quantities of water, to change the respiratory gradient in their leaves. Only a few exploratory experiments have been performed on this subject. It is probable, as has been pointed out earlier (9), that some of the secondary changes in the respiratory gradient during ontogeny may be due to increases in the soil water content rather than to ageing. No experiments on the standard leaves have been carried out to investigate the effects of increasing the soil water content after the plants have reached the 13-day-old stage. A few experiments have been made on the effects of withholding water from plants which have been grown to the 13-day-old stage with a water supply of 60 ml. daily.

TABLE II

THE EFFECT OF WITHHOLDING WATER FROM "STANDARD" WHEAT PLANTS ON THE "STABLIZED" RATES OF OXYGEN UPTAKE IN THE LEAVES

Days without water	Age in days	Respiration rate, mgm. 0_2 per 100 gm. fresh weight				
		Quarter 1	Quarter 2	Quarter 3	Quarter 4	Whole leaf
0	13	21.0	20.7	23.1	19.1	21.0
1	14	23.8	21.9	20.1	16.5	20.7
2	15	23.1	18.4	19.6	16.2	19.3
3	16	19.9	18.2	16.2	14.9	17.3
5	18	18.7	15.2	17.7	14.0	16.4
5	18	22.5	14.4	17.1	18.4	17.7
7	20	38.1	—	30.2	30.4	—

In the experiments on the effects of withholding water from the plants, a group of plants were grown to the 13 day stage, and then no more water was added to the cans in which the plants were growing. Samples were taken after 12 hr. of illumination, on Day 13 and subsequent days during the following week. Since all the cans did not contain the same number of plants, the wilting did not progress at quite the same speed in all the cans. Among the results given in Tables II and III, this fact shows in the two samples which had had five days without water. The second of these samples represents a more advanced stage in wilting than the first one. The leaves of the samples down to and including the first of the five day wilted samples were turgid. In the second of the five day wilted samples the upper halves of the leaves were flaccid at the time of gathering, but they rapidly gained water when their ligule ends were placed in water prior to quartering. The water content values for all these samples are too high since they were all placed in water immediately after removal from the plants and before quartering. This had been adopted as the standard procedure for the respiration determinations. The leaves from the cans with no water for seven days varied from flaccid in their upper halves to flaccid in all quarters. The data in Table II show that as the soil water decreases, the respiration rates of the quarters decrease to a minimum and then rise rapidly when the water content becomes so low in the soil that the plants wilt extensively during the period of illumination. This behavior is similar to that of the plants in the soil water series, as can be seen from Fig. 1. No very great changes occur in the respiratory gradient during wilting, until the leaves become flaccid during the periods of illumination. When the leaves start suffering from severe water deficits, the respiration rate in the fourth quarter rises above that in the third quarter as is the case in the 15 ml. water daily plants. In the wilting series, samples after one and three days without added water show a uniform gradient in respiration from the tip to the base of the leaves, a condition not to be found in any of the leaves of plants from the soil water series which had had small water supplies. With this exception the gradient behavior is similar to that of the plants of the soil water series.

TABLE III

THE EFFECT OF WITHHOLDING WATER FROM STANDARD WHEAT PLANTS ON THE STABLIZED DRY WEIGHT RATES OF OXYGEN UPTAKE IN THE LEAVES

Days without water	Age in days	Respiration rate, mgm. 0_2 per 100 gm. dry weight				
		Quarter 1	Quarter 2	Quarter 3	Quarter 4	Whole leaf
0	13	206	232	256	222	228
1	14	224	242	239	192	225
2	15	234	206	218	190	214
3	16	207	206	178	183	207
5	18	183	173	194	167	182
5	18	216	169	184	200	192
7	20	280	—	298	310	—

The data in Table III show that when water is withheld from the plants, the effects on the respiration rate, calculated on the basis of dry weight, are the same as the effects of growing the plants under progressively drier conditions from the start, except in the case of extreme drought (15 ml. daily and seven days wilted). In the case of wilted plants, the rise in dry weight respiration when the wilting becomes severe is much greater than in the case of plants grown with low soil moisture.

Except under very dry conditions, the gradients in dry weight respiration rates are similar regardless of whether the shortage of water has developed after the plants reached the 13 day stage or whether it was present all the time that the plants were growing. This indicates that the gradient is certainly not referable to the anatomical stucture of the leaf alone, but to physiological considerations, at least in part. The effects of varying soil water do not arise from changes in the anatomy of the leaf at some critical stage in the development of the young leaf, but must be caused by the effect of changed conditions on the mature leaf which has a fixed anatomical structure.

The air lines of starving quarters of the soil water and wilting series of experiments do not differ appreciably from those of the standard leaves described earlier (12).

Changes in Water Content

Fig. 3 shows that wheat leaves grown in cans supplied 60 ml. of water daily have the greatest water content. If more water is added each day then the water content drops slowly with increasing water supplied. A possible explanation for this will be considered later. As the water supplied falls below 60 ml. per day the water content drops. This drop in water content becomes quite rapid when the water supply is low. No doubt the low water content of leaves of plants grown in dry soil is a result of the physical difficulty of removing water from a soil which contains very little water.

Fig. 4 shows that in all plants supplied with 45 ml. of water or more per day there is a gradient in water content from the base to the tip of the leaves, with the greatest amount of water in the basal quarter of the leaves. This gradient is more or less obliterated in the leaves of plants supplied with 30 ml. of water per day. With plants grown under conditions of severe drought the water content falls quite low and the gradient becomes irregular, with sister samples of leaves showing different gradients. Two cases out of three showed a gradient which was opposite to the normal gradient which latter shows most water in the basal quarter. This is thought to be the result of the protein gradient in which the most protein is found in the tip quarters. When the water content becomes low the tendency of the protein to bind water begins to affect the distribution of water in the leaves. The normal water content gradient is probably the result of the fact that the basal quarter is nearer to

the roots and consequently nearer to its water supply than is the tip quarter and so it would be expected to contain the greatest proportion of water assuming substantial uniformity in water loss over the whole leaf surface.

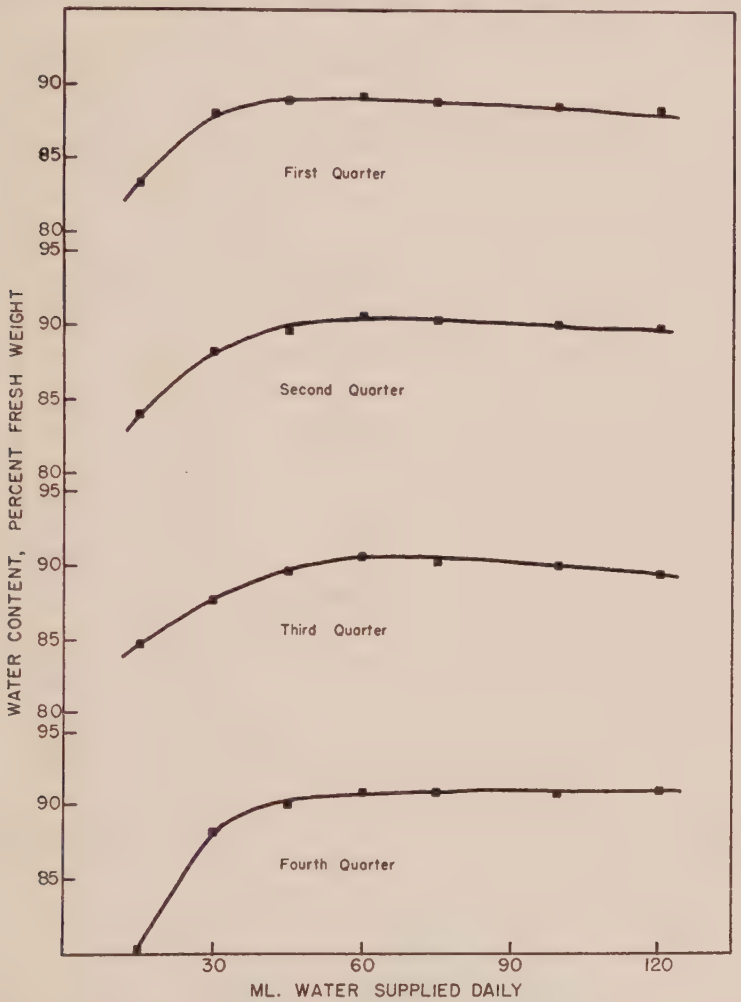


FIG. 3. The changes in the water content of the four quarters of 13-day-old wheat leaves induced by different daily water supplies. The leaves were harvested after one to two hours of darkness.

The data in Table IV show that in the early stages of the wilting experiments, the water contents of the quarters of the leaves tend to rise slightly. This surprising condition will be considered later. When, however, the soil water supply becomes very low the leaves wilt and their water content falls as one would expect. No great changes occur in the water gradient in the leaves during the drop in soil water until the water available falls so low that the leaves wilt and even then the change is not very great.

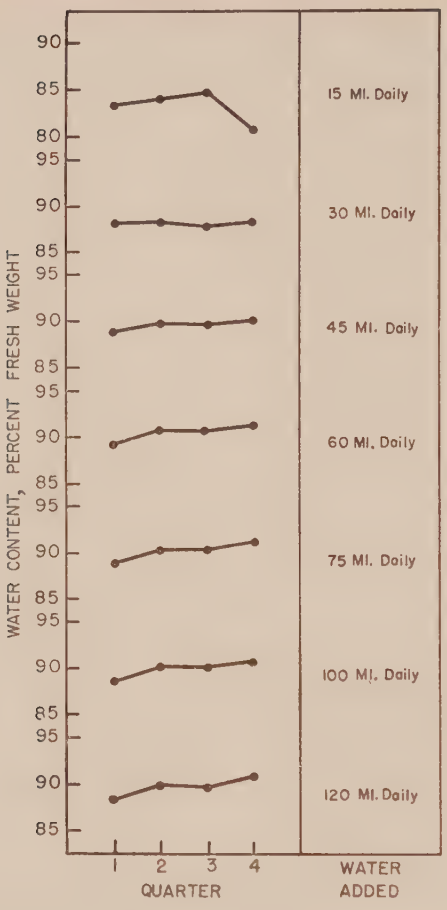


FIG. 4. The gradients in water content of wheat leaves from plants supplied with various amounts of water daily and harvested after one to two hours of darkness on the 13th day after seeding.

TABLE IV

THE EFFECT OF WITHHOLDING WATER FROM STANDARD WHEAT PLANTS ON THE WATER CONTENTS OF THE LEAVES

Days without water	Age in days	Water content as percentage fresh weight				
		Quarter 1	Quarter 2	Quarter 3	Quarter 4	Whole leaf
0	13	89.8	91.1	91.0	91.4	90.8
1	14	89.4	90.95	90.6	91.4	90.6
2	15	90.1	91.1	91.0	91.5	90.95
3	16	90.4	92.15	91.4	91.85	91.45
5	18	89.8	91.2	90.9	91.6	91.0
5	18	89.6	91.5	90.7	90.8	90.75
7	20	86.4	90.8	89.9	90.2	89.7

Changes in Nitrogenous Substances

Protein Nitrogen

Regardless of whether the protein nitrogen content is calculated on the basis of fresh or dry weight there is a strong protein gradient in the leaves. This gradient does not appear to be much affected by the water content of the soil in which the plants were grown. Hence the respiration rate cannot be controlled by the protein gradient alone since it has already been shown that the respiratory gradient can be modified by changing the water content of the soil in which the plants are grown.

Table V shows that plants grown in dry soil have a relatively greater amount of protein per unit fresh weight than do plants grown in wetter soil. This is, however, largely the result of the lower water content of plants grown in dry soil. These points will be reconsidered when the general effects of wet and dry soil on the growth of wheat plants are considered.

TABLE V

THE EFFECTS OF THE SUPPLY OF SOIL WATER ON THE PROTEIN NITROGEN CONTENTS OF THE FIRST LEAF OF WHEAT

Soil water supplied daily, ml.	Protein nitrogen content as percentage fresh weight				
	Quarter 1	Quarter 2	Quarter 3	Quarter 4	Whole leaf
30	0.519 0.504	0.481 0.483	0.444 0.504	0.338 0.376	0.440 0.465
60	0.420 0.433	0.352 0.379	0.301 0.308	0.231 0.258	0.338 0.349
100	0.410 0.366	0.348 0.351	0.317 0.324	— 0.233	— 0.323

Soluble Nitrogen

Table VI shows that there is a well marked gradient in soluble nitrogen in wheat leaves. Normally this gradient is fairly uniform from base to tip of

TABLE VI

THE EFFECTS OF THE SUPPLY OF SOIL WATER ON THE SOLUBLE NITROGEN CONTENTS OF THE FIRST LEAF OF WHEAT

Soil water supplied daily, ml.	Soluble nitrogen content as percentage fresh weight				
	Quarter 1	Quarter 2	Quarter 3	Quarter 4	Whole leaf
30	0.356 0.451	0.431 0.385	0.473 0.379	0.390 0.347	0.418 0.386
60	0.160 0.175	0.222 0.191	0.251 0.232	0.257 0.242	0.220 0.207
100	0.151 0.156	0.167 0.202	0.205 0.198	0.192 0.208	0.178 0.193

the leaves, with greater percentage of soluble nitrogen in the basal quarter of the leaves. It appears that this gradient may be reversed under dry conditions. Whether the soluble nitrogen content is calculated on the basis of fresh weight or dry weight there is no doubt that plants grown under dry conditions contain larger quantities of water soluble nitrogenous compounds per unit mass than plants grown in moister soil. A possible explanation for this will be considered subsequently.

Changes in Reducing Substances

Studies of the reducing power of extracts of wheat leaves determined by various methods following various treatments (10) indicate that in wheat leaves the free reducing sugars constitute at most a small proportion of the whole. The true identity of these reducing substances is unknown. Whatever these substances are, they show a prominent gradient in the leaves from tip to base with the greatest quantity in the tip quarter (see Table VII).

TABLE VII

THE DISTRIBUTION OF REDUCING SUBSTANCES CALCULATED AS GLUCOSE IN THE FIRST LEAF OF WHEAT PLANTS GROWN WITH DIFFERENT DAILY WATER SUPPLIES

Soil water added daily, ml.	Reducing substances as glucose as percentage of fresh weight									
	Quarter								Whole leaf	
	1		2		3		4			
	H	S	H	S	H	S	H	S	H	S
30 (4 hr. D)	0.76	0.64	0.47	0.35	0.32	0.22	0.28	0.13	0.47	0.39
	0.96	0.69	0.76	0.69	0.56	0.43	0.38	0.26	0.69	0.54
60 (0 hr. D)	0.27	0.17	0.18	0.09	0.16	0.08	0.14	0.07	0.21	0.09
	0.26	0.18	0.16	0.09	0.13	0.06	0.09	0.06	0.17	0.07
100 (4 hr. D)	0.34	0.21	0.21	0.16	0.20	0.11	—	—	—	—
	0.38	0.23	0.27	0.17	0.22	0.12	0.20	0.07	0.27	0.13

NOTE: *D* = *Hours of darkness before harvest.*
H = *Hanes' method.*
S = *Somogyi method.*

There is decidedly more of the material in the plants grown in dry soil than there is in those grown in moist soil. Judging from the gradient in reducing substance and its behavior under the influence of changes in soil moisture, it cannot be responsible for the changes in the respiratory gradient which occur with changing soil moisture.

Changes in Sucrose

In an earlier publication (10) the conclusion was reached that sucrose was the only nonstructural carbohydrate present in the young wheat leaves grown

in the greenhouse in December. It is possible that, under the different conditions of culture in the growing chambers, other carbohydrates may be present in the leaves.

If fructosans, maltose, or dextrins were present in the leaves their presence should be indicated by either or both the invertase and fructose residue determinations. No indications of the presence of these substances were obtained.

The values for sucrose, based on the determination of fructose residues, tend to be lower than the values obtained by the other methods. This is

TABLE VIII

THE DISTRIBUTION OF SUCROSE CALCULATED ON A FRESH WEIGHT BASIS IN THE LEAVES OF WHEAT

Soil water added, ml.*	Method used**	Sucrose as percentage fresh and dry weight									
		Quarter 1		Quarter 2		Quarter 3		Quarter 4		Whole leaf	
		D.W.	F.W.	D.W.	F.W.	D.W.	F.W.	D.W.	F.W.	D.W.	F.W.
30 (4 hr. D)	H	5.1	0.61	6.1	0.71	4.6	0.55	2.9	0.33	4.8	0.56
	S	5.3	0.64	6.3	0.73	4.9	0.59	3.7	0.42	5.1	0.59
	I	5.3	0.64	6.3	0.73	4.8	0.58	3.6	0.41	5.1	0.60
	F	4.6	0.55	3.6	0.42	3.4	0.40	3.5	0.40	3.9	0.46
30 (4 hr. D)	H	5.4	0.71	5.9	0.72	4.8	0.61	2.9	0.34	5.0	0.62
	S	4.0	0.52	4.7	0.57	3.8	0.48	0.0	0.00	3.4	0.42
	I	5.0	0.66	5.1	0.62	3.8	0.48	2.3	0.27	4.2	0.52
	F	5.3	0.70	5.3	0.65	2.3	0.29	1.1	0.13	3.8	0.47
60 (0 hr. D)	H	8.1	0.91	7.0	0.70	5.9	0.57	5.3	0.49	6.8	0.68
	S	8.7	0.98	7.2	0.72	6.2	0.60	3.4	0.31	6.2	0.62
	I	8.1	0.91	7.2	0.72	6.4	0.62	4.3	0.39	6.3	0.63
	F	7.3	0.82	5.0	0.50	3.6	0.35	1.4	0.13	4.7	0.47
60 (0 hr. D)	H	7.4	0.84	8.1	0.83	6.0	0.61	4.0	0.37	6.8	0.68
	S	8.2	0.93	8.6	0.87	6.1	0.62	4.1	0.38	7.2	0.72
	I	8.2	0.93	7.9	0.80	5.9	0.60	4.0	0.37	7.0	0.70
	F	6.4	0.72	6.8	0.69	4.2	0.42	1.8	0.17	5.3	0.53
100 (4 hr. D)	H	6.7	0.65	5.8	0.54	5.7	0.52	—	—	—	—
	S	7.8	0.76	5.4	0.50	5.6	0.51	—	—	—	—
	I	6.3	0.62	5.8	0.54	5.1	0.46	3.8	0.31	5.3	0.49
	F	5.4	0.53	4.5	0.42	4.6	0.42	0.5	0.04	4.9	0.45
100 (4 hr. D)	H	4.9	0.51	4.1	0.37	3.0	0.28	2.8	0.23	3.8	0.35
	S	4.4	0.46	3.1	0.28	3.2	0.30	2.8	0.23	3.5	0.33
	I	4.8	0.50	3.6	0.33	3.1	0.29	3.0	0.25	3.7	0.34
	F	4.6	0.48	3.3	0.30	3.4	0.32	2.1	0.17	3.9	0.36

** Numbers in brackets indicate the number of hours of darkness to which the plants had been subjected before harvest.*

*** H values obtained by Hanes' method after acid hydrolysis.*

S values obtained by Somogyi's method after acid hydrolysis.

I values obtained by Somogyi's method after invertase hydrolysis.

F values obtained on the assumption that all the fructose residues determined by a modification of Roe's method are present in the form of sucrose.

F.W. Sucrose as per cent fresh weight.

D.W. Sucrose as per cent dry weight.

doubtless on account of the presence of interfering substances whose effects are only approximately eliminated by the correction which was applied in an effort to eliminate the effects of the interference.

Comparing similar quarters, and allowing for the rapid loss of sucrose known to occur during the early hours of darkness, the data in Table VIII show that there is very little difference in the sugar content of leaves of plants grown with different daily water supplies. The gradients in sucrose calculated on the basis of dry weight are similar to those calculated on the basis of fresh weight. With two exceptions, there is a gradient in sucrose from the tip to the base of the leaves with most sucrose in the tip quarter of the leaves. This gradient is probably associated with the chlorophyll gradient in the leaves. The latter gradient can be clearly demonstrated by soaking equal weights of the different quarters in equal volumes of alcohol and observing the intensity of the green color in the alcohol. There is most chlorophyll in the tip quarter and least in the basal quarter. In plants grown with a water supply of 30 ml. per day the tip two quarters of the leaves contain approximately equal percentages of sugar. In the 100 ml. of water daily plants the sugar contents of the second and third quarters are approximately equal. When these gradients are compared with the respiratory gradients, it is seen that the agreement is poor except in the case of plants grown with 100 ml. of water added per day.

Discussion

Consequences of Varying Soil Water Supply

In addition to yielding valuable information about the respiratory gradient and its relationship to the other gradients in the leaf, the set of experiments on soil moisture has brought out two additional features of interest. The first is that the first leaves of wheat seedlings from plants grown in dry soil contain a higher concentration of nitrogenous substances per unit mass than do those of plants grown in wetter soil. The second point is that there is an optimum soil water level as far as producing wheat leaf tissues with a high water content is concerned.

The nitrogen per unit mass of leaf tissue in the plants grown in soil supplied with only 30 ml. of water per day is greater than in any of the other plants tested. However, the average quantity of nitrogen per leaf is greatest in the case of the leaves of plants supplied with 60 ml. of water per day, as is shown in Table IX. This situation arises from the much smaller size of the leaves of the plants grown in the drier soil.

In spite of the fact that the grains of Khapli wheat contain approximately 1.4 mgm. of nitrogen each it is probable that a considerable fraction of the nitrogen in the first leaf must be absorbed from the soil. Unless there is a very great difference between the nitrogen distribution among the plant organs of plants grown in dry soil and the organs of plants grown in moister soil, the plants grown in dry soil must absorb much more mineral nitrogen per unit weight than those grown in wetter soil. Now a considerable amount of evidence has been accumulated to show that the uptake of ions by the roots

TABLE IX

TOTAL NITROGEN PER FIRST LEAF IN MGM.

	Water supplied daily, ml.		
	30	60	100
Experiment 1	0.47	0.58	0.52
Experiment 2	0.55	0.59	0.51

of plants is associated with aerobic respiration. This has been shown to be the case by Hoagland and Broyer for barley roots (4). In this paper many of the references to earlier work on *Nitella* and *Valonia* are given. Further confirmation of this conclusion has been obtained by Machlis (8) who demonstrated that several respiratory inhibitors inhibit salt uptake while malate, citrate, succinate, and fumarate increase the salt uptake of barley roots. As the soil moisture is increased, the aeration of the soil becomes poorer. Thus the supply of oxygen available for root respiration decreases. It is probable that the large quantities of mineral salts absorbed by the plants in the dry soils are a result of the large supply of oxygen available for the aerobic respiration of the roots in these dry soils. It is probable that the changes in the soil water supplied exert part of their effect on the plants by changing the amounts of mineral salts that the plants can extract from a given soil.

The data already presented show that plants grown in wet soils have lower water contents in their leaves than plants grown in moist soils. Since all conditions except soil water were the same for the different sets of plants in the soil water series of experiments, the conclusion must be drawn that plants grown in wet soils find it more difficult to absorb water from the wet soils than the plants grown in moist soils find it to obtain their water. The mineral content of the wet soils will not be as great as that in the dry soils, as some leaching of the mineral matter from the cans occurs where very large quantities of water are given daily. Furthermore, the concentration of the soil water solution in the wet cans will be lower than in the moist or dry cans. On these grounds it would be expected that the plants in the wet soil would have the greatest water content. It is apparent that excessive soil moisture, probably operating through partial root suffocation, lowers the ability of the plants to absorb water from the soil. The effect of root suffocation may be direct in that the uptake of water from the soil may be dependent on respiratory activity in much the same way as in the case of salt uptake but to a smaller degree. The other possibility is that root suffocation causes some other detrimental changes to occur in the roots which lower their ability to absorb water. Such a change could be lower salt concentration in the root hair cells induced by the root suffocation. The slight rise in the water contents of plants which had their soil water supply diminished by withholding water also indicates that better root aeration results in increased ability to absorb water from the

soil. It shows that the residual bad effects of mild root suffocation can soon be overcome by plants. This experiment does not enable a decision to be reached as to whether soil aeration directly affects the ability of the roots to absorb water, or whether it exerts its effects through its direct influence on some other process such as salt uptake or growth. This question is in great need of experimental clarification.

That the absorption of water by plants is retarded in a poorly aerated soil has been frequently observed. Hoagland (3) and many others have called attention to the fact that continued absorption of water can proceed only when conditions are favorable to aerobic respiration. Henderson (2) showed a close correlation between the rates of water absorption by corn roots and their respiration as measured by carbon dioxide or oxygen exchanged with the surrounding fluids. Absorption of unit volume of nutrient solution with an osmotic pressure of 1.8 atmospheres was accompanied by the absorption of twice as much oxygen as absorption from distilled water. In addition Van Overbeck (15) and Rosene (14) have shown that a large portion of the water absorption of roots can be reversibly inhibited by potassium cyanide in concentrations that are known to inhibit a similar fraction of aerobic respiration. Also Kramer (6) and Hoagland and Broyer (5) found that high concentrations of carbon dioxide around roots abruptly decreased water uptake. Although these effects of respiratory inhibitors strongly suggest a direct connection between respiration and water uptake, this point is not settled at present. The retardation in the rate of root growth due to poor aeration in the soil undoubtedly has an important effect on the ability of plants to absorb water as suggested by Kramer and Coile (7).

The effects of root suffocation should be considered in conjunction with the respiratory correlatives of cellular elongation in leaves which will be discussed in a later paper (see 9). In this connection it should be borne in mind that during elongation it is probable that the cells take up a good deal of mineral material, and it is certain that they absorb a great deal of water in addition to the other processes which they carry on at that time. During this stage of their development, they take up huge quantities of oxygen. It is possible that this is in order to release the energy for salt and water accumulation.

The Basis of the Physiological Heterogeneity

The gradients in protein nitrogen, soluble nitrogen, reducing substance, and sucrose in the first leaf are apparently little affected by the soil water supplied to the plants. On the other hand the soil water supply appreciably affects the respiratory gradient. It is therefore apparent that the distribution of none of these substances completely controls the respiration rate in any one part of the leaves. A similar conclusion has been reached with respect to the changes in the respiratory rate and the gradient in the first leaf under changing durations of illumination and darkness prior to harvest (13).

In a previous paper it was suggested that leaf anatomy was an important factor in the cause of the respiratory gradient. Considerable changes can be

induced in the respiratory gradient under conditions which cannot possibly be associated with anatomical changes. Such conditions are prolonged darkness and the withholding of water from plants after the first leaf has reached maturity. However when it is suggested that there are anatomical differences between different parts of the leaf it is implied that there are enzymic differences between different parts of the leaf. If it is assumed that the different cell types in the leaf have differences in their metabolic pathways then it is to be expected that different conditions will affect the different cell types differently. Slight differences in R.Q. between the different quarters have been observed (9) and these differences indicate that there are quantitative differences between the amount of material turned over in the different metabolic pathways in the different quarters. Since the leaf can obtain its water minerals and other solids only through one end of the leaf whereas gaseous exchange can take place through stomata scattered over the leaf surface then there must be differences in the cellular environments in different parts of the leaf. This is reflected in the water content gradient. This may also cause cell types with similar enzymic complements to behave differently in different parts of the leaf. Probably all the differences between the different quarters of the leaves are to be explained by the combined effects of differences in anatomy, cellular environment, and the enzymic machinery in the different parts of the leaf.

Acknowledgments

I should like to express my appreciation to Professors G. H. Duff and D. F. Forward for their valuable criticisms and suggestions which have been made throughout the course of this study.

References

1. Duff, G. H. and Forward, D. F. Physiological and biochemical studies in plant metabolism. I. The respiration of the seedling wheat leaf in starvation and ontogeny. Can. J. Research, C, 27 : 125-145. 1949.
2. Henderson, L. Relation between root respiration and absorption. Plant Physiol. 9 : 283-300. 1934.
3. Hoagland, D. R. Lectures on the inorganic nutrition of plants. Chronica Botanica, 1944.
4. Hoagland, D. R. and Broyer, T. C. General nature of the process of salt accumulation by roots with description of experimental methods. Plant Physiol. 11 : 471-507. 1936.
5. Hoagland, D. R. and Broyer, T. C. Accumulation of salt and permeability in plant cells. J. Gen. Physiol. 25 : 865-880. 1942.
6. Kramer, P. J. Causes of decreased absorption by plants in poorly aerated media. Am. J. Botany, 27 : 216-220. 1940.
7. Kramer, P. J. and Coile, T. S. An estimate of the volume of water made available by root extension. Plant Physiol. 15 : 743-747. 1940.
8. Machlis, L. Influence of some respiratory inhibitors and intermediates on respiration and salt accumulation of excised barley roots. Am. J. Botany, 31 : 183-192. 1944.
9. Roberts, D. W. A. The metabolic basis of physiological heterogeneity in the cells of a linear organ. Ph.D. Thesis, University of Toronto. 1948.
10. Roberts, D. W. A. Qualitative and quantitative determination of the wheat leaf carbohydrates. Can. J. Research, C, 28 : 754-779. 1950.

11. ROBERTS, D. W. A. Nitrogen determination in wheat leaves. Can. J. Research, C, 28 : 745-753. 1950.

12. ROBERTS, D. W. A. Physiological and biochemical studies in plant metabolism. III. The effects of starvation and mechanical stimulation on the respiratory metabolism of the first leaf of the wheat plant. Can. J. Botany, 29 : 383-402. 1951.

13. ROBERTS, D. W. A. Physiological and biochemical studies in plant metabolism. IV. Effect of illumination and darkness on the physiological heterogeneity in the first leaf of wheat. Can. J. Botany, 29 : 451-466. 1951.

14. ROSENE, H. F. Effect of cyanide on root exudation in excised onion roots. Am. J. Botany, 31 : 172-174. 1944.

15. VAN OVERBECK, J. Water uptake by excised root systems of tomato due to non osmotic forces. Am. J. Botany 29 : 677-682. 1942.

SEASONAL VARIATION IN THE CHEMICAL COMPOSITION OF THE *FUCACEAE* IN THE MARITIME PROVINCES[1]

By Margaret G. Macpherson and E. Gordon Young

Abstract

Fucus vesiculosus, *F. evanescens*, and *Ascophyllum nodosum* have been analyzed monthly for their content of moisture, mineral salts, organic nitrogen, mannitol, laminarin, and alginate over a period of two years. Plants were collected from two localities, St. Andrews, N.B., and Halifax, N.S. In general, when the ash content was at a minimum in the winter months, alginate was at a maximum. The converse was true in spring and summer. Organic nitrogen and laminarin remained relatively constant throughout the year. Mannitol was highest in the summer and autumn, fluctuating with the temperature of the water. No essential difference was detected between the results of St. Andrews and at Halifax, or between the three species examined. Analyses carried out on samples collected at different times on the same day showed no significant differences.

In the past, the biochemistry of seaweeds has been neglected for the most part except for occasional analysis directed to particular constituents, such as iodine and potassium, when these substances were in special industrial demand. The marine algae, both *Rhodophyceae* and *Phaeophyceae*, present interesting problems because of their unusual and distinctive chemical composition, and their unusual habitat. The sublittoral flora, especially the *Laminariaceae*, have received more attention because of size and potential industrial uses. In the eastern maritime provinces the great abundance of the *Fucaceae* and of *Chondrus crispus* makes it desirable that more should be known about them. These plants occur in a more or less definite position in the littoral zone of the shore and present a fascinating problem of physiological adaptation and of transition from sea to land.

Investigations of the chemical composition of the *Fucaceae* have been rather limited. Some comparative analyses have been made by Kylin (13, 14), Hendrick (12), Hass and Hill (10, 11), Colin and Ricard (9), and on eastern Canadian material by Butler (6) and Macpherson and Young (17).

Differences in composition of different parts of the plant have been established in *Fucus vesiculosus* by Moss (18). The apex contained more mineral salts, mannitol, laminarin, and alginate than the base. Differences between fruiting receptacles and sterile tips were not marked but the latter tended to have more mannitol and a higher content of total solids (Moss (19)).

Studies on the variation in composition with season of the year and in different habitats have now been completed in Scotland at the Gulland Institute on *Ascophyllum nodosum* (1) and on *F. serratus*, *F. vesiculosus*. *F. spiralis*, and *Pelvetia canaliculata* (3). The only comparable investigation on the Canadian east coast was done by Butler (7) on *Chondrus crispus*, in

[1] *Manuscript received October 22, 1951.*

Contribution from The Department of Biochemistry, Dalhousie University, Halifax, N.S., and The Nova Scotia Research Foundation.

which it was shown that the total carbohydrate and ash rose in the spring and summer months and the organic nitrogen fell.

In general, the ash, nitrogen, iodine, and alginate of littoral seaweeds were at a *maximum* in the *winter* months while laminarin and mannitol were at a minimum (1). The ash content was relatively constant between 14 and 24% of dry weight. There was a marked seasonal variation in the nitrogen content (0.4-2.4%) which reached its maximum in March or April in all species and its minimum in the early winter months (Oct.-Dec.). This may be related to the exhaustion of inorganic nitrate in the surrounding water (5). Little seasonal variation in alginate has been observed (24-30%). Degree of exposure to wave action affected the composition in various ways (4), as did also depth of immersion (2).

In the frond, mannitol was at a minimum when the new ones were developing and before photosynthesis had developed to any degree. Synthesis of mannitol and laminarin reached a peak in midsummer or later. Alginate metabolism was the converse of this cycle. The *Fucaceae* differ from the *Laminariaceae* in that alginate is present in higher concentration in the former and laminarin is always present (4, 15).

Over the past two years we have carried out monthly analyses for moisture, mineral salts, organic nitrogen, mannitol, laminarin, and alginate on three species, *F. vesiculosus*, *F. evanescens*, and *A. nodosum*, in two localities in the Maritime Provinces, St. Andrews, N.B., and Halifax, N.S.

Experimental

Preparation of Material

The plants were collected at low tide from the same area about the 15th of each month from July, 1949, till July, 1951. They were transported directly to the laboratory and analysis commenced immediately. Several plants were incorporated in each sample. The habitat may be described as sheltered with little exposure to wave action, at both St. Andrews, N.B., and Halifax, N.S.

For moisture determination the plants were shaken and blotted to remove most of the surface moisture. The stipes were removed from the specimens of *Fucus* but not of *Ascophyllum*. Most of the material was then dried in an oven at 100° C. for 48 hr. The dried fronds were ground by passage through a Wiley mill and screened through a No. 20 wire mesh. This material was kept in stoppered bottles. It was later dried to constant weight before analysis, or the moisture content was determined. Plants collected at St. Andrews were dried before shipment to Halifax. No determinations of total moisture were therefore made on this material.

Methods

Moisture determinations were done on 20 to 40 gm. of the fresh plants by drying to constant weight at 100° C.

The total ash content was found by igniting 1 to 2 gm. in an electric muffle furnace at 575° to 600° C. This required about 20 hr.

TABLE I

CHEMICAL COMPOSITION OF THE *Fucaceae* AS PERCENTAGE OF DRY WEIGHT OF THE PLANT, EXCEPT FOR THE MOISTURE DETERMINATIONS

Species and locality	Moisture, %		Total ash, %		Mannitol, %		Laminarin, %		Alginate, %		Nitrogen, %	
	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
A. nodosum												
Nova Scotia	69.4	80.5	14.0	31.4	3.8	10.1	1.7	5.2	5.0	20.6	1.6	2.8
New Brunswick	—	—	19.1	24.4	5.8	9.4	2.9	5.5	9.0	18.8	1.0	1.8
Scotland*	66.0	79.0	14.0	26.0	7.0	12.0	2.5	7.5	24.0	28.0	0.4	1.7
F. vesiculosus												
Nova Scotia	73.8	83.5	16.4	26.7	5.6	12.8	1.6	3.4	6.8	16.3	1.6	3.5
New Brunswick	—	—	13.3	28.6	6.7	11.0	2.5	6.7	7.5	15.0	1.0	2.4
Scotland**	64.3	81.0	13.8	20.7	8.3	16.1	2.0	4.9	13.8	17.2	0.9	1.8
F. evanescens												
Nova Scotia	76.1	88.4	15.1	25.1	4.4	12.3	2.5	5.4	6.6	16.9	1.7	3.2
New Brunswick	—	—	16.3	25.9	5.6	10.9	2.3	4.7	7.5	16.2	1.1	2.5

* *Black (1).*
** *Black (3).*

Total organic nitrogen was estimated by the usual Kjeldahl–Gunning method.

Mannitol, laminarin, and alginate were determined by the methods developed by Cameron, Ross, and Percival (8) and they are the same as those used by Black (1, 2, 3, 4).

Results

The minimum and maximum values obtained are presented in Table I for both localities together with the results of Black (1, 3) for areas in Scotland. No differences are apparent in the quantitative levels of the fluctuations in the Canadian species examined. Compared with the Scottish results it may be concluded that the fluctuations are of the same order, except in the case of alginate in which the Canadian plants fall to a much lower minimum, especially in *A. nodosum*.

Table II presents the yearly average figure for the constituents examined in relation to other values in the literature. Such figures however are now only significant if they represent comparable seasonal periods. The agreement however is good.

TABLE II

ANNUAL AVERAGES OF CHEMICAL CONSTITUENTS AS PERCENTAGES OF THE DRY WEIGHT, EXCEPT FOR MOISTURE DETERMINATIONS

Species and locality	Moisture, %	Total ash, %	Alginic acid, %	Laminarin, %	Mannitol, %	Organic nitrogen, %
A. nodosum						
Nova Scotia	75.1	19.1	14.4	3.1	8.1	1.9
New Brunswick	—	21.2	14.0	4.0	7.4	1.2
Nova Scotia*	67.3	19.8	—	—	—	1.8
Nova Scotia††	70.0	22.2	—	—	—	—
Norway***	—	21.0	26.0	2.0	5.0	1.3-2.4
France†	—	—	18.6	4.3	8.9	—
F. vesiculosus						
Nova Scotia	77.1	20.3	13.3	2.7	10.1	2.0
New Brunswick	—	23.3	11.7	3.2	8.6	1.5
Nova Scotia*	76.6	21.5	—	—	—	1.7
Nova Scotia††	76.4	20.7	—	—	—	1.4
Scotland**	72.7	18.3	15.5	3.5	12.1	1.3
Norway***	—	22.0	19.0	2.0	5.0	1.4-2.9
F. evanescens						
Nova Scotia	82.1	20.6	13.1	3.4	8.7	2.1
New Brunswick	—	22.0	11.5	3.1	9.1	1.8
Nova Scotia*	86.5	17.7	—	—	—	2.7

* *Macpherson and Young (17) for collection in May.*
** *Black (3) on annual basis.*
*** *Lunde (16).*
† *Colin and Ricard (9) for collection in September.*
†† *Butler (6) for collection in November.*

The variation in water temperature at St. Andrews and at Halifax is shown in Fig. 1. The detailed analytical observations are presented in Figs. 2 to 7. In general it may be concluded that the three species examined exhibited the

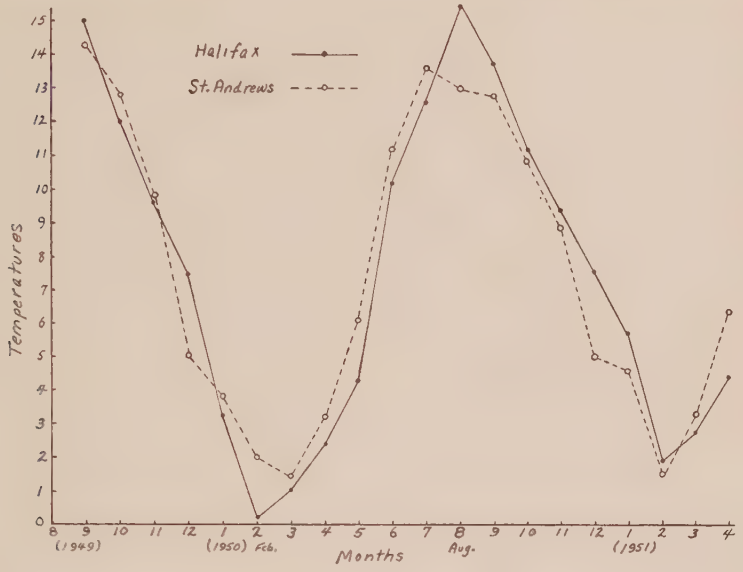


FIG. 1. Seasonal variation in the temperature of the water at Halifax, N.S., and at St. Andrews, N.B., 1949-51.

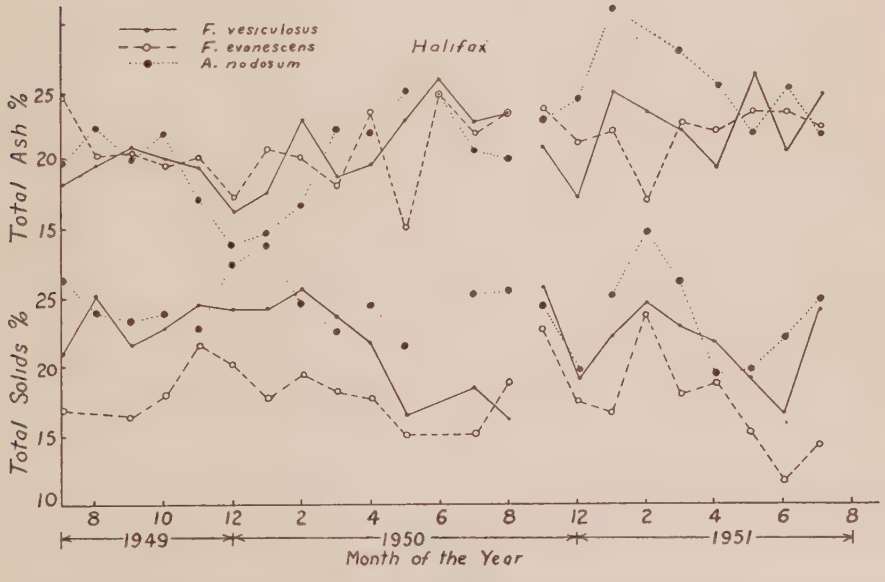


FIG. 2. Seasonal variation in composition of total ash and of total solids at Halifax, N.S., 1949-51.

same variations and that there were no differences detectable between the results at St. Andrews and at Halifax. The figures for nitrogen are not presented in graphic form because of their relative constancy. With the

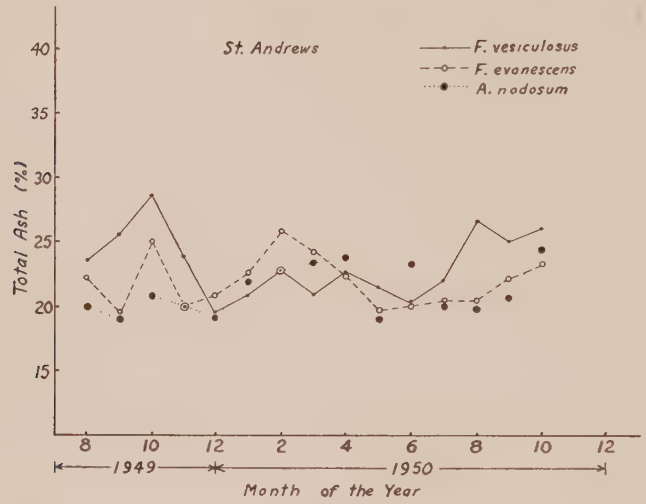


FIG. 3. Seasonal variation in composition of total ash at St. Andrews, N.B., 1949-50, as percentage of total solids.

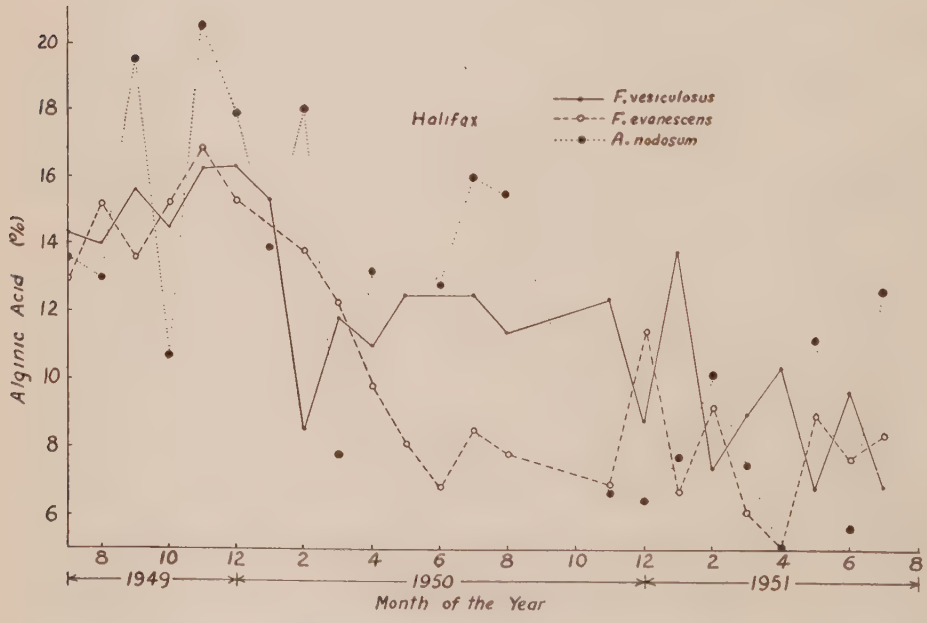


FIG. 4. Seasonal variation in composition of alginic acid at Halifax, N.S., 1949-51, as percentage of total solids.

exception of the total solids, all results are expressed on the basis of percentage of the anhydrous plant. There are both advantages and disadvantages to this practice.

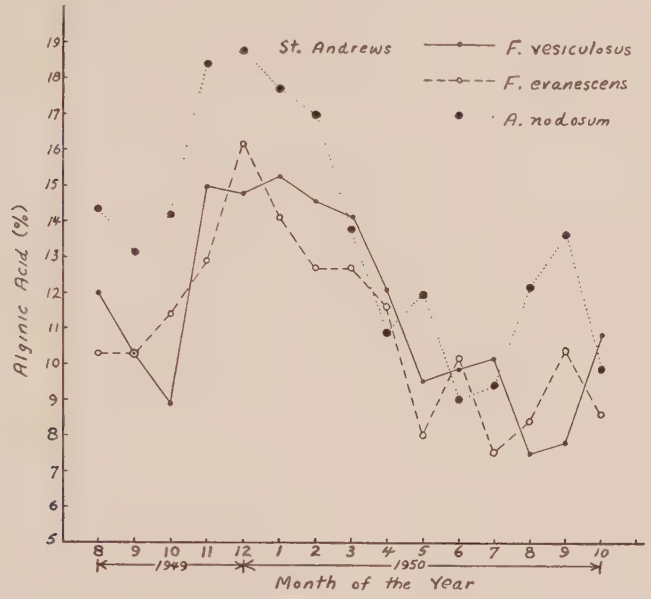


FIG. 5. Seasonal variation in composition of alginic acid at St. Andrews, N.B., 1949-50, as percentage of total solids.

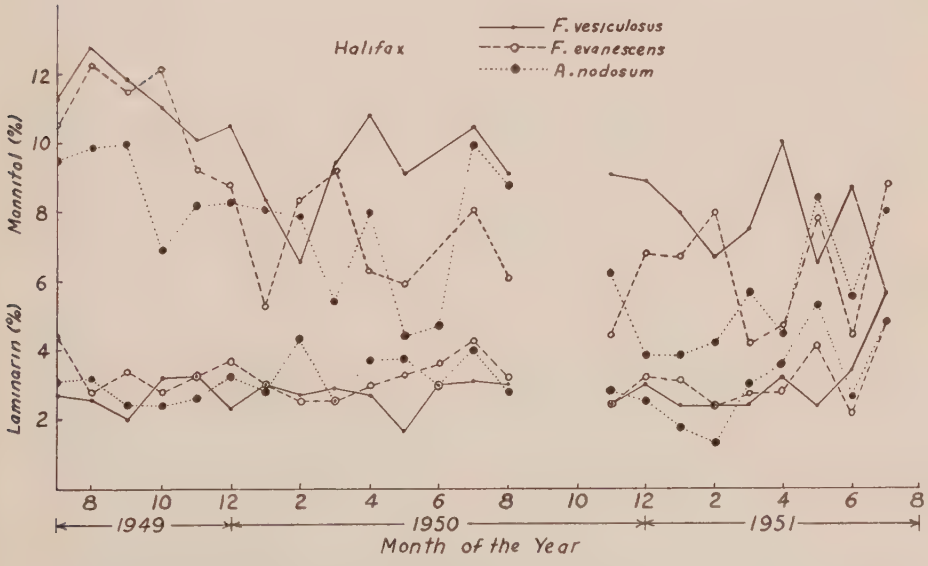


FIG. 6. Seasonal variation in composition of laminarin and of mannitol at Halifax, N.S., 1949-51, as percentages of total solids.

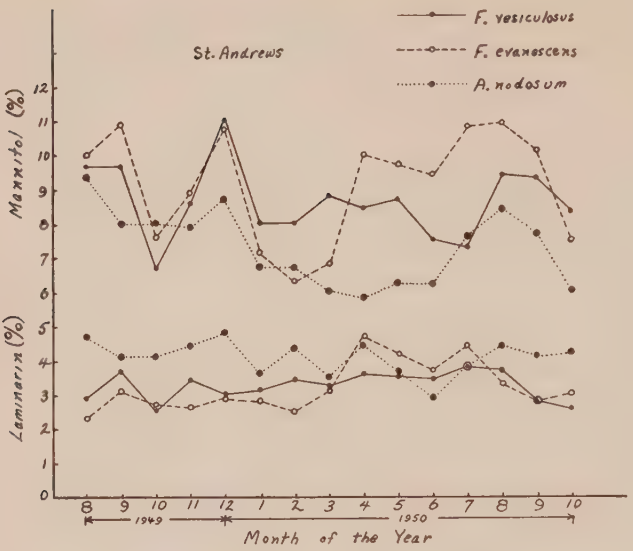


FIG. 7. Seasonal variation in composition of laminarin and of mannitol at St. Andrews, N.B., 1949-50, as percentages of total solids.

Moisture.—The water content was at a minimum during the winter months and at a maximum in the spring in conformity with land plants.

Total ash.—From the graphs it appears, surprisingly, that in 1949-50 all species exhibited minima in the winter months from November to February and maxima in the spring and autumn at Halifax. The results at St. Andrews were rather constant, fluctuating only between 19 and 27%. The observations for Halifax specimens in 1951 showed maxima in the winter months.

Alginic acid.—Maximum values were observed between November and March after which a precipitous fall took place to minimum levels during May till July or August. The Halifax results show marked fluctuations and are not consistent from year to year.

Laminarin.—Both at St. Andrews and Halifax the fluctuation was from about 2 to 4% and no specific seasonal variation was apparent, unlike the previous results of Black (1, 3).

Mannitol.—Definite fluctuations were shown in this important constituent. From maximum figures of 10 to 13% attained in the spring and summer the levels fell gradually in the autumn to low points of 5 to 7% during the period of colder water. The levels for *A. nodosum* appeared to be lower than for *Fucus*. There may have been a minor secondary rise about November.

Nitrogen.—The fluctuations of organic nitrogen were comparatively small. The maximum value of about 3% was reached in February, March, and April in all species, and the minimum value of about 1% in August, September, and October. These observations are in agreement with those of Black.

Variation in Composition During One Day

Collection of specimens for the analyses recorded above was always done on the 15th of the month at about noon. Some of the variations observed might have been due to a *daily* fluctuation in composition in response to intensity of photosynthesis, or to different degrees of desiccation on tidal exposure. We have therefore carried out analyses on specimens of *F. vesiculosus* collected at different times on the same day and, in consequence, at different depths of submergence. The results are presented in Table III.

TABLE III

VARIATION IN COMPOSITION OF *F. vesiculosus* DURING THE DAY

Date and constituent	Time, condition, and concentration (%)			
	8:30 a.m.	11:30 a.m.	2:30 p.m.	6:30 p.m.
	Exposed, rainy	Exposed, rainy	Submerged, rainy	
VI. 15, 51				
Moisture	84.4	83.4	89.3	
Mannitol*	8.5	8.7	8.5	
Laminarin*	2.3	3.4	2.9	
	Exposed, foggy	Exposed, sunny	Submerged, sunny	Submerged, sunny
VII. 14, 51				
Moisture	76.7	75.8	75.5	79.2
Mannitol*	9.0	5.6	8.7	7.8
Laminarin*	2.6	2.2	2.3	2.3

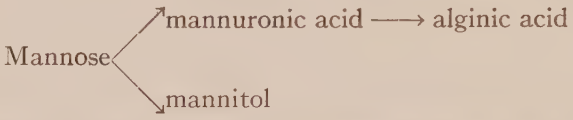
* *Expressed as percentage of dry weight.*

They do not show any appreciable fluctuation in composition that cannot be attributed to experimental variation. The unexpected value of 5.6% for mannitol on July 14 was rechecked so that it is not an analytical error, and is the converse of what would be expected from increased metabolic activity.

Discussion

Several factors undoubtedly play a part in the causation of the fluctuations in the composition of marine algae. The fundamental one is metabolic activity but this is probably controlled by the temperature of the surrounding water, the concentration of essential nutrients, and the intensity of light. The age of the plant appears to be a minor factor. Black and Dewar (5) have clearly demonstrated the importance of the concentration of essential nutrients, such as nitrate, phosphate, and oxygen, in the surrounding water. Habitat, such as depth of immersion, is also important, probably in relation to intensity of light (Black (2)) and desiccation. All of these factors vary with the season of the year which makes a clear analysis of related phenomena very difficult.

Ricard (20) associated metabolic activity in *Laminaria* with temperature of the sea. Concentration of laminarin, which may be considered as algal "starch", was his index. The synthesis of laminarin from mannitol seems probable and these two substances are often in reciprocal relationship. In our observations they have changed concentration levels however in parallel. This may mean a photosynthesis of mannitol which is more rapid than the condensation to laminarin in the *Fucaceae*. Mannitol may also be related to the synthesis of alginic acid via mannose as follows:



The concentration of total ash must be related to the base-binding capacity of the total solids present. In the *Fucaceae* these include alginic acid as a polymer of D-mannuronic acid and fucoidin, as a fucosan ethereal sulphate. Both total ash and alginate show considerable fluctuation but parallelism is not evident in our observations. No analyses for fucoidin have been made to determine the importance of this factor. The function of alginate has not been definitely established but it appears to act as a structural element and possibly as a buffer against desiccation and changes in osmotic pressure. It is a powerful hydrophilic colloid. In our observations alginate was high in the winter months and low in the spring, in converse relationship to the temperature of the water.

The nitrogen values include a variety of substances, at present only partially identified in the fucoids, including protein, polypeptides, amino acids, and amines. Black (1) observed wide fluctuations in organic nitrogen in which a sharp maximum was noted in the early spring (March) when the inorganic nitrate of the sea water was at a maximum. The same period for maximum levels occurred in our analyses of organic nitrogen but the fluctuation was more gradual and not as great. This aspect of algal metabolism deserves further investigation.

Acknowledgments

We wish to take this opportunity of expressing our grateful thanks to Mr. L. R. Day of the Atlantic Biological Station, St. Andrews, N.B., for providing the samples from that region. We also acknowledge our indebtedness to the Nova Scotia Research Foundation for financial assistance which made this investigation possible.

References

1. Black, W. A. P. The seasonal variation in the chemical composition of some of the littoral seaweeds common to Scotland. I. *Ascophyllum nodosum*. J. Soc. Chem. Ind. (London), 67 : 355-357. 1948.
2. Black, W. A. P. Seasonal variation in chemical constitution of some common British Laminariales. Nature, 161 : 174. 1948.
3. Black, W. A. P. The seasonal variation in the chemical composition of some of the littoral seaweeds common to Scotland. II. *Fucus serratus*, *F. vesiculosus*, *F. spiralis* and *Pelvetia canaliculata*. J. Soc. Chem. Ind. (London), 68 : 183-189. 1949.

4. Black, W. A. P. The seasonal variation in weight and chemical composition of the common British Laminariaceae. J. Marine Biol. Assoc. United Kingdom, 29 : 45-72. 1950.

5. Black, W. A. P. and Dewar, E. T. Correlation of some of the physical and chemical properties of the sea with the chemical constitution of the algae. J. Marine Biol. Assoc. United Kingdom, 28 : 673-699. 1949.

6. Butler, M. Comparison of the chemical composition of some marine algae. Plant Physiol. 6 : 295-305. 1931.

7. Butler, M. Seasonal variations in *Chondrus crispus*. Biochem. J. 30 : 1338-1344. 1936.

8. Cameron, M. C., Ross, A. G., and Percival, E. G. V. Methods for the routine estimation of mannitol, alginic acid, and combined fucose in seaweeds. J. Soc. Chem. Ind. (London), 67 : 161-164. 1948.

9. Colin, H. and Ricard, P. Glucides et dérivés glucidiques des algues brunes. Compt. rend. 190 : 1514-1516. 1930.

10. Haas, P. and Hill, T. G. An examination of the metabolic products of certain fucoids. I. Sugar. II. Mannitol and mannitan. Biochem. J. 23 : 1000-1004; 1005-1009. 1929.

11. Haas, P. and Hill, T. G. A preliminary note on the nitrogen metabolism of seaweeds. Glutamic acid peptide. Biochem. J. 25 : 1472-1475. 1931.

12. Hendrick, J. The value of seaweeds as raw materials for chemical industry. J. Soc. Chem. Ind. (London), 30 : 565-574. 1916.

13. Kylin, H. Zur biochemie der meeresalgen. Z. physiol. Chem. 83 : 171-197. 1913.

14. Kylin, H. Untersuchungen über die biochemie der meeresalgen. Z. physiol. Chem. 94 : 337-425. 1915.

15. Lunde, G. Seaweed as a source of raw materials. Angew. Chem. 50 : 731-734. 1937. (*From* Chem. Abstracts, 31 : 8747. 1937.)

16. Lunde, G. Utilization of seaweed and kelp as fodder. Skand. vet.-tid. bakt. patol. kött-och mjölkhygien, 30 : 26-62. 1940. (*From* Chem. Abstracts, 34 : 3393. 1940.)

17. Macpherson, M. G. and Young, E. G. The chemical composition of marine algae. Can. J. Research, C, 27 : 73-77. 1949.

18. Moss, B. Studies in the genus *Fucus*. I. On the structure and chemical composition of *F. vesiculosus* from three Scottish localities. Ann. Botany, 12 : 267-279. 1948.

19. Moss, B. Studies in the genus *Fucus*. II. Anatomical structure and chemical composition of receptacles of *F. vesiculosus* from three contrasting habitats. Ann. Botany, 14 : 396-410. 1950.

20. Ricard, P. Les constituants glucidiques des Laminaires: nature, variations saisonnières. Bull. soc. chim. biol. 13 : 417-435. 1931.

A SURVEY OF CERTAIN SEAWEEDS OF COMMERCIAL IMPORTANCE IN SOUTHWEST NOVA SCOTIA[1]

By Constance MacFarlane[2]

Abstract

The Nova Scotia Research Foundation is making an extensive survey of seaweeds of the Province that are of potential commercial importance. The area in which the survey has been completed extends from Cape Sable Island, Shelburne County to Chebogue, Yarmouth County, and includes also a small part of Digby County. The survey includes both quantitative measurements and biological studies of fucoids, *Laminaria* spp., and *Chondrus*, in all of which the region abounds. Of 325 miles of coastline surveyed for rockweed, 140 miles are harvestable, bearing approximately 200,000 tons. Results obtained at 255 stations, examined in detail, showed the average density to be 37 lb. per sq. yd. The width of the zone is from 2 to 300 yd., the average being 50 yd. *Ascophyllum nodosum* forms the greatest percentage of rockweed, *Fucus vesiculosus* ranking next. *Fucus serratus* is recorded for the first time in this part of the Province. *Laminaria* beds totalling 12,000 ac. in extent and bearing 900,000 tons were charted. Mortality among *Laminaria* sporophytes is high and the number in a bed varies from year to year. In 1950 7,997,739 lb. of *Chondrus* were harvested. Density varies from 1 to 2½ lb. per sq. ft. *Chondrus* beds are listed and their ecology discussed. Recolonization of denuded areas is described and succession of algal cover noted on denuded areas and on concrete blocks placed in *Chondrus* and *Laminaria* beds. The survey region is compared with regions surveyed in Scotland. A map of the survey region is included.

Introduction

For the purpose of obtaining quantitative information regarding the commercial possibilities of marine algae of Nova Scotia, the Nova Scotia Research Foundation for the past three summers has been conducting an extensive survey on the southwest shores of the Province, including portions of Shelburne and Yarmouth Counties. During the summers of 1948, 1949, and 1950 the survey has been carried on chiefly in these two counties, and has been concerned with three types of algae, namely the fucoids, or rockweeds; the laminarias, or kelps; and *Chondrus*, or Irish moss. Observations on these algae have been made at stations in the area centered around Pubnico. Although the investigation is not yet complete, there has been an increasing demand for information, and as very few quantitative records are available for seaweed growth (3, 4, 6, 7, 9, 18, 20, 24, 25, 26), it was deemed desirable to place on record the information obtained to date.

The accompanying map shows the area covered, and indicates representative stations investigated.

Choice of Location

The reason for initation of the survey in this area was that already there was developing a small industry in Irish moss, employing a goodly number of lobster fishermen during their free summer months. The choice of location

[1] *Manuscript received October 24, 1951.*
Contribution from the Nova Scotia Research Foundation, Halifax, N.S.

[2] *Algologist.*

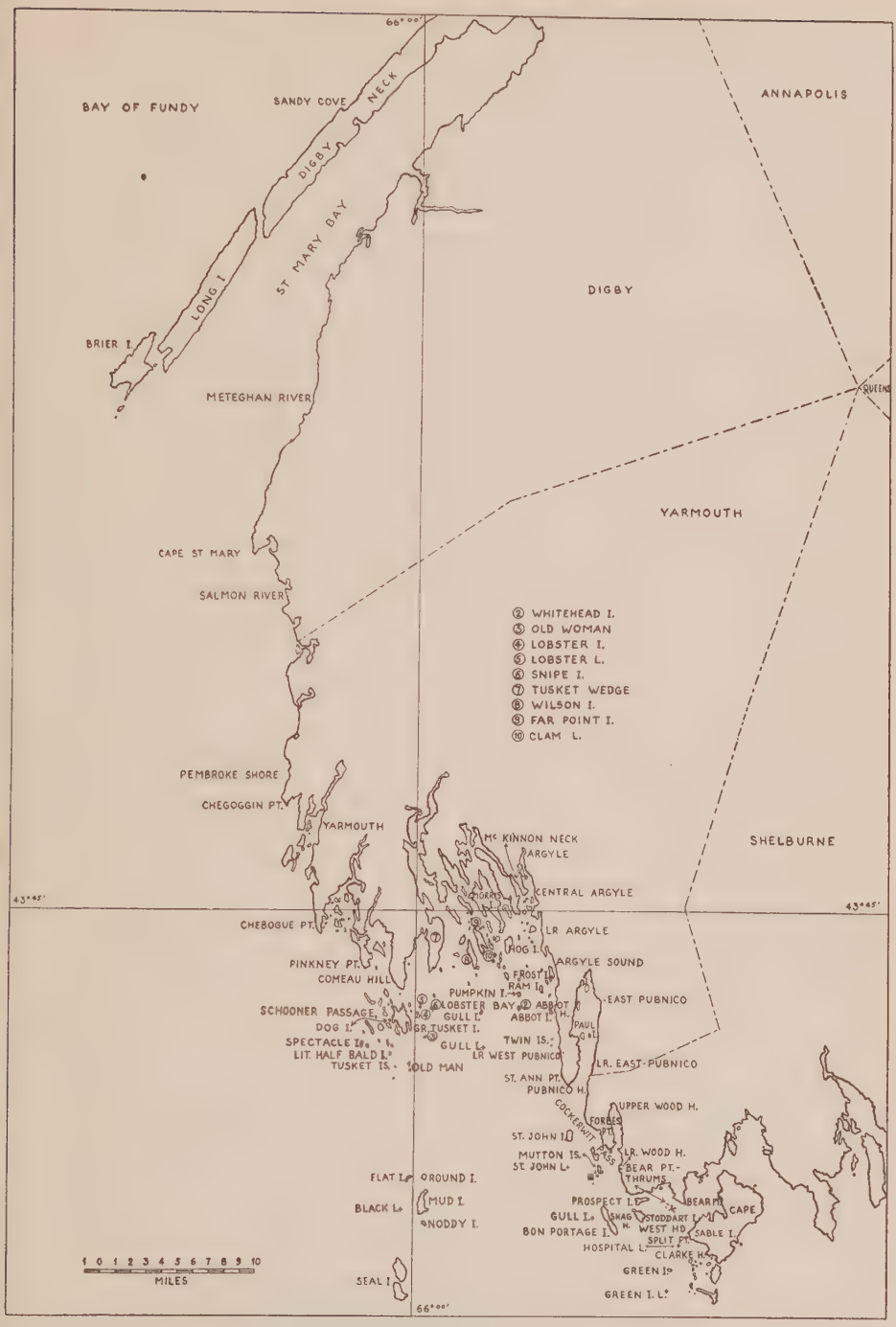


FIG. 1 COAST OF SOUTHWEST NOVA SCOTIA SHOWING AREA SURVEYED FOR ROCKWEEDS, KELPS AND IRISH MOSS.

FIG. 1.

was a particularly happy one, situated as it is in the Fundy tidal area, with its extensive intertidal zone and long sloping shoreline supporting luxuriant growth in both the littoral and sublittoral zones.

The region is of interest not only because of its broad littoral zone, caused by the great Fundy tides and the gently shelving shore, but also because of the rock formation of the bottom, and the tremendous stirring of the Fundy waters. Because of its geological origin and the unceasing action of tides and currents, the bottom of the survey region is ever undergoing constant change. At present it is uneven and varied, consisting as it does of a series of drowned river beds with many small islands still remaining above sea level, and, where they are sufficiently worn away, former islands are now replaced by numerous bars, reefs, and ledges, most of which form good attachment for beds of *Chondrus* and *Laminaria*. In addition to these there are a great many shallow areas where the bottom consists of small stones or gravel and shells, and frequently of living *Mytilus* upon all of which may be found fairly large quantities of *Laminaria longicruris*. Where the islands, chiefly drumlins (10), remain, they are usually surrounded by a narrow intertidal rim, either muddy or rocky and supporting *Ascophyllum* and *Fucus*. Beyond this is a narrow sublittoral fringe which, provided it is not muddy, usually is covered with *Chondrus* and *Laminaria* before descending into deeper water with a muddy or sandy bottom.

As is indicated by the geological maps (8), the Atlantic side of Shelburne–Yarmouth area has a rock formation of quartzite and granite which provides suitable anchorage for large seaweeds – a condition differing markedly from the basalt columns and the softer sandstone inside the Bay itself where the larger seaweeds are known to occur only in restricted sheltered spots. The contrast in the two types of substrata with their different resultant weed cover provides a study of great interest. The writer hopes to deal with the ecological aspects in a subsequent paper.

The omission of this interesting region from the survey by Bell and MacFarlane in 1933 (1, 2) made it all the more necessary to deal with it in detail during the present survey.

Methods and Equipment

A 40-ft. "Cape Island" fishing boat was rented each season and a smaller (14 ft.) flat-bottomed boat with outboard motor was built for special work. It could be taken into shallow areas inaccessible to the larger motor boat and, in addition, by saving time otherwise required for rowing, made it possible to observe a more extensive area during a single low tide.

As far as was possible, detailed measurements were made of the extent of the area, the percentage of cover, and the weight of cover per unit area. This last was done sometimes by random sampling, sometimes by more accurate measurements of complete strips. In the case of the rockweeds and *Chondrus*, the strips were made through the length of the intertidal zone. In the case of the laminarias, less regular 'strips' were made by cutting a series of samples

at short regular intervals across the length and breadth of shoals offering conditions suitable for this procedure. Where such conditions were not present, such as in beds following the shoreline of certain islands, random samples were taken to give sufficient indication of the growth per unit area. The area was then calculated, and its total weight of weed determined.

When conditions did not permit such detailed analysis, typical sites were selected for which accurate measurements were made. The quantity of weed was then estimated on the basis of the measured sites.

A. *Rockweeds*

As no detailed geological maps (1 mi = 1 in.) are available for the area under consideration, Admiralty charts and topographical maps were consulted before making any exploratory studies. All accessible regions were subsequently studied at low water, some being reached by boat and others overland.

At each place visited, a 25 or 36 sq. ft. quadrat typical of the vicinity was selected and the weed cut off with a knife at a point from one to three inches distal to the holdfast. The weed was placed in a wire basket, weighed immediately with a spring balance, and the weight recorded. The accuracy of the spring balance was checked weekly. The weight of weed per sq. yd. was calculated, the percentage cover estimated, and the average density thus obtained for each locality.

The width of the rockweed band (i.e. the distance between upper and lower limits of harvestable weed) was measured by means of a calibrated rope. The length of the corresponding shoreline was estimated and checked with calculations obtained from Admiralty charts, aerial photographs, and maps. From these data the amounts of weed in the various localities were estimated.

At the upper limits of the intertidal zone there usually occur *Fucus* plants too short to be of interest to a commercial harvester. These plants were not included in measurements of width or determinations of weight.

Detailed rockweed maps of the area were prepared from aerial photographs and field notes. The maps are on file at the offices of the Research Foundation in Halifax. It is expected that these will be published at a later date. The present report, being a preliminary one, does not include details of maps or of statistics.

B. *Kelps*

Locating *Laminaria* beds and assessing their available tonnage presented problems not encountered in the survey of the littoral weeds. During the first part of the survey, no suitable means was available to obtain the density except in extremely shallow water where quadrats could be cut by hand. Even locating the beds necessitated good weather and fairly calm water.

The success of *Laminaria* investigation is affected by the tide. The best time for sampling is governed by the strength of the current even more than by the actual height of water overlying the beds. The Fundy tidal currents, which are equally strong at great depths and on the surface, turn in direction

on the surface and below at almost the same time (22). The velocity of the current and the actual time of slack water, however, vary with the location. In channels and passages between islands, there is often a difference between the center and the sides of 30 min. to an hour in the time of slack water.

This characteristic of the current is useful to surveyors as it enables them to operate for a longer period than would otherwise be possible. The average length of time for satisfactory operation is during the period between two hours before and two hours after the time of low water slack. At other times the laminarias are carried downward to the bottom by the force of the current and are not easily detached. Moreover, in strong currents it is not possible to operate the boat and gear with any success.

Owing to the inequalities of the substrate, already described, the method of bottom survey used by the Scottish Seaweed Research Association, i.e. by transects (24, 25), was not suitable for this region. Instead, various means were used to locate beds and measure their size and density – the means depending on the type of bed and on the facilities available. The various methods of location and assessment of density are listed below.

1. Beds in very shallow water

During low water of spring tides many beds could be noted by the presence of curved stipes projecting above the surface in the more shallow portions of the area. Quadrats of 36 sq. ft. were cut by hand with a Scottish sickle, from a dory, and weighed with a beam balance. This method is always used in regions too shallow for safe operation of the motor boat.

2. Beds in less shallow water

Laminarias can often be detected on calm sunny days by their color as seen through the water. Their presence was frequently confirmed by observation through a view box, constructed of wood or metal, the bottom being of glass and the sides tapering to a narrow opening at the top. The narrowing of the opening prevented the light from entering at the top and thus improved the visibility.

For determining densities in such depths the Scottish sickle was sometimes used. Sickle measurements, however, are time-consuming, and unless the current is too strong or the growth of the weed too heavy, grab measurements are to be preferred.

In 1949, from blueprints sent through the courtesy of the Scottish Seaweed Research Association, there was constructed a modified Peterson grab which cuts off the growth from $\frac{1}{2}$ sq. yd. of substratum. The grab was put into operation in June 1950. In 1951 a few alterations were made to improve its efficiency in cutting the strong stipes of *Laminaria digitata* too often ripped through or left uncut. Except in regions of excessively heavy growth, where the grab cannot contain all the plants growing on $\frac{1}{2}$ sq. yd. of substrate, this piece of equipment has proved most satisfactory and has greatly facilitated the sublittoral survey.

3. Beds in water deeper than $2\frac{1}{2}$ to 3 fathoms

The heavy tide rips and strong Fundy currents cause a general turbidity in the waters throughout the whole region, rendering the view box practically useless on very dark days and even on fine days through layers of water deeper than $2\frac{1}{2}$ to 3 fathoms. For location of beds in such places as this, and for determining the edge of a *Laminaria* stand, a lightweight drag was useful. It was constructed of galvanized pipe and rods with teeth so arranged as to catch in plants no matter which side fell to the bottom. The drag was operated by hand from the motor boat and could be used in most weathers. A greased sounding lead also indicated the presence or absence of weed beds and was useful in locating their outer limits.

4. Regions of doubtful nature

In many deeper regions the substratum and cover were doubtful and the tidal currents even at low water too strong for satisfactory operation of the grab. In these instances, in order to ascertain with certainty the presence or absence of laminarias, a robot camera designed for underwater photography was found to be of great value. Photoflash bulbs, used as the source of light, gave excellent results, most of the pictures being sharp and clear. For more extensive photography of sea bottom, a battery of photoflood lamps would probably be more satisfactory as it would give a series of consecutive pictures each time the camera is lowered. Such a battery has been used recently by Vevers (23) off the south coast of England.

For measuring the areas of beds, a number of methods were used.

1. The outer limits of certain small beds were marked with lobster pot buoys, the distance between measured with a calibrated cod line, and the area calculated.

2. If such areas were close to a shore offering space sufficient for a suitable baseline, a prismatic compass was used and the area calculated by triangulation.

3. Most beds were plotted directly on an Admiralty chart, and the area measured with a planimeter. This method is the one now in general use and is satisfactory for all but the smallest beds.

Although all these methods are still used to locate the beds and estimate their available tonnage, the general procedure now usually followed is first to consult Admiralty charts for likely places. The depth and type of bottom of the selected area is then found with a sounding lead and the presence or absence of laminarias, as described above, is subsequently determined with the drag or the grab. The rope of the grab is calibrated to check the depth. A large number or random samples are taken to find the average density and percentage cover. Each area is plotted and its tonnage estimated after planimeter measurements are obtained from the chart.

The eastern portion of the survey was made easier by the use of aerial photographs specially prepared through the courtesy of the Royal Canadian

Air Force, who used methods already devised by British workers. The photographs indicate the presence of weeds and also assist in estimating the percentage cover (5).

Although the procedure followed does not give the location of all the available *Laminaria*, it is considered satisfactory in that it gives the best results for the best areas where weed cover occurs in quantities sufficient for scythe-harvesting and grab-sampling. Because of the varying conditions of the bottom, there are some areas for which no quantitative data were determined. In many of the heavier tide rips not only is maneuvering the boat dangerous, but satisfactory operation of the grab is impossible. Still other areas support only an intermittent growth of laminarias. Situations of these types are suitable only for dragging and grapnel harvesting, and although they would yield many tons, they are not included in this report. It must be understood, therefore, that the estimated weights of *Laminaria* given in this paper are for "beds" only.

C. *Irish Moss*

Most of the *Chondrus* beds were already known to resident lobster fishermen who harvest the weed from dories on calm days in summer. All the 'mossing' grounds familiar to the harvesters were visited and quadrats of 1 sq. ft. were sampled for condition of beds and their density. The areas of the beds were estimated and the width possible for harvesting by existing methods measured. Additional beds were sometimes discovered. The location of *Chondrus* at deeper levels was noted, and weights per unit area recorded when conditions permitted.

A rake similar to that used by the harvesters was employed to sample the bottom for relative percentage of *Chondrus* and extraneous weeds. In deeper waters grab samples of $\frac{1}{2}$ sq. yd. were obtained. When the *Chondrus* was exposed at low water of spring tides, quadrats of 1 sq. ft. were cut by hand. At other times none of these methods of sampling was possible as then not only is the *Chondrus* too far below the surface for quadrat cutting, but continuous surf and ground swell prevent all but intermittent raking, and always render it impossible to make exact measurements.

Owing to the impracticability of adequate measurement, it was not possible to make a statistical survey of areas and tons of *Chondrus*. Instead, the Irish moss brokers were approached early in the 1950 season and their aid solicited. At the end of the season with the assistance of the brokers, the writer completed forms giving weekly information on weather conditions, 'quality' of moss, number of dories used, and the amount of *Chondrus* harvested in the various localities.

Results of Survey

A. *Rockweeds*

Species Occurring in the Survey Area

Ascophyllum nodosum (L.) LeJol. forms the bulk of the easily harvestable rockweed in this part of Nova Scotia.

Ascophyllum Mackaii (Turn.) Holmes and Batt. occurs in fair quantities in many marshy places, but is unlikely to be used in any large commercial quantity, its only such use at present being for packing worms shipped for bait.

Fucus vesiculosus L. is found throughout the whole region, growing both above and below the limits of the *Ascophyllum*.

Fucus evanescens C. Ag. is commonly found on the islands at low water level. On the mainland it is of infrequent occurrence in the eastern sections of the survey area but occurs to some extent further west, increasing in frequency towards Cheboque and Yarmouth.

Fucus edentatus De la Pyl. is commonly found on the islands and is quite abundant on shoals and ledges exposed at low water. St. John's Ledge, south of Pubnico Point contains these last two species in abundance, as do the islands further out to sea, notably Seal Island, and the Bald Tuskets.

Fucus serratus L. previously recorded for Nova Scotia only in Northumberland Strait and near Cape North in Cape Breton, has not been seen to date in Shelburne County nor in the eastern part of Yarmouth County presently under observation, but among the Tusket Islands it occurs in considerable quantities particularly at The Candlebox, Owl's Head, and other places in Schooner Passage as well as at Seal Island, the Tuskets, and the islands just east of Tusket Wedge.

Fucus spiralis L. occurring in sheltered regions, usually above *F. vesiculosus*. Of no commercial significance.

Fucus filiformis Gmelin, observed only in upper tide pools at St. Ann Point. Of no commercial significance.

Fucus, though found generally throughout the region, forms only a small percentage of the cover in the fucoid zone which is dominated by *Ascophyllum nodosum*.

Extent of Survey

The rockweed survey extended from Cape Sable Island westward to Chebogue. It included all mainland shores, as well as Cape Sable Island, Bon Portage, the Mutton Islands and associated ledges, all the islands and ledges in Lobster Bay, the Tusket Islands, Mud Island, Seal Island, and two stations near Cape St. Mary's. Sampling and/or measurements and observations were made at 255 stations where the shoreline supported rockweed in harvestable quantity. Table I below shows the density and estimated weight of some of the best locations for harvesting.

Measurements taken at all 255 stations indicate that for the whole region surveyed the average density on rockweed-bearing areas is 37 lb. per sq. yd. Of 325 miles surveyed, the extent of harvestable shore line on the mainland, islands, and ledges was found to be 140 miles. The width of the rockweed band varies according to the locality from 2 yd. to 300 yd., the average width being about 50 yd. In the area under study there are approximately 200,000 tons of harvestable rockweed (fresh weight). In a similar survey in Scotland 4250 miles of coast was shown to bear 180,000 tons (20, 25).

TABLE I

ROCKWEED AREAS OF HEAVY DENSITY (85-100% COVERED)

Area	Density in lb. sq. yd.	Width, yd.	Length, yd.	Estimated weight, tons
Abbot Harbour	38	75-250	440	1400
Tusket Wedge	55	30-150	8000	11,500
Dog Island and Ledges	20	110	660	700
Near Clarke Harbour, Cape Sable Island	36	150-200	3000	8000
Frost Island Ledges	22	35- 85	2600	11,000
Argyle Sound and Lower Argyle	45	25-100	4400	6400
Hog Island, South End	22	40-100	1000	800
Forbes Point	36	40-300	5280	15,000
N. Mutton Island	59	30-110	3500	6500
Hay Island and Ledges	58	30- 90	1300	1800
Islands in Mudflats west of Lobster Ledges	50	50	220	275
Clam Ledge	32	115	300	500

The "density" is the average found in each vicinity.

The width of the band varies with the shoreline and substratum, and is usually irregular in any given area.

"Length" refers to distance along the shoreline or ledge.

The outer Tusket Islands, and such places as Seal Island, the Mud Islands group, and the west coast of St. John Island are greatly exposed to the prevailing westerly winds. The rockweed of their shores is negligible compared with that on such semiprotected areas as Tusket Wedge, Abbot Harbour, and Egg Island. The great width of the rocky intertidal zone combined with excessive aeration of the Fundy waters and partial shelter from the full force of the Atlantic storms all result in conditions favorable for the growth of rockweed.

B. *Kelps*

Species Occurring in the Survey Area

Laminaria digitata (L.) Edmonson is found generally throughout the district, in exposed situations. A form which may be *L. intermedia* f. *cucullata* Foslie is also found in somewhat sheltered localities where silt is often carried by the ebbing currents of the region. It is possible that such plants are growth forms of *L. digitata*, their morphological peculiarity being an ecological manifestation.

Laminaria Agardhii Kjell. Although common further east in the Province, only three specimens fulfilling the specifications descriptive of the species were observed during the present survey. In 1948 one plant was found off Snipe Island and two in the channel between Far Point Island and Morris Island.

Laminaria longicruris De la Pyl. Several ecological forms occur here, the size and form varying with the location. This is the dominant species in most areas, and occurs generally throughout the region. A detailed account of this *Laminaria* will appear in a separate paper. (Taylor's note on *L. faeroensis* (21) is of interest in this connection as is also the description of *L. saccharina* in the comprehensive article by Parke (17).)

Alaria esculenta (L.) Grev. In large numbers on Black Ledge and St. John Ledge and in lesser quantities in other exposed regions. As it is of no present commercial significance, and does not occur in commercial quantities, no attempt was made to assess the tonnage.

Agarum cribrosum (Mert) Bory. Commonly found at Seal Island, Bon Portage, St. John Ledge. Occurring occasionally elsewhere; quantity insufficient to assay weights.

Phyllaria dermatodea (De la Pyl.) Le Jol. Found in large numbers on a ledge in southwest part of Pubnico Harbour; elsewhere only scattered plants.

Extent of Survey

Of the area surveyed, over 12,000 ac. were found to consist of "beds" of harvestable *Laminaria*. The density varied from 26 to 128 tons per ac. and the total available weed (the sum of the individual beds) was estimated at 900,000 tons. Maps of the *Laminaria* beds are on file at the Foundation office.

The heaviest plants of *L. longicruris* grown in the swift currents on shoals and reefs west of Schooner Passage, at Peases Island Ledge, Old Man, Flat Island, Seal Island, and off Cape Sable Island. The heaviest growth of *L. digitata* occurs in the waters of the Bald Tuskets, Seal Island, Bramble Island Shoal, Jones' Ledge, Ram Island, St. John Ledge, Goodwin Island Bar, Robinson's Bar, Gull Island Bar, and near Cape Sable Island on Hospital Reef, Pork Ledge, Green Island, Little Green Island, and Seal Rocks.

Tables II and III show typical *Laminaria* beds of contrasting densities.

TABLE II

AREAS AND ESTIMATED WEIGHTS OF TYPICAL REGIONS DENSELY POPULATED WITH *Laminaria*

Locality	Av. density, lb./sq. yd.	Area, ac.	Estimated weight, tons
St. John Ledge	34	350	28,800
Twin Islands and adjacent shoals	40	70	6800
Abbot Island	43	55	5700
Ram Island, Tarrio Ledge, and adjacent ledges	35	125	10,500
Jones Ledge	35	60	5000
Pumpkin Island and Ledge	35	160	13,500
Whitehead Island and West Shoal	38	50	4600
Old Man	22	225	12,000
Old Woman Shoal	52	200	25,100
Little Half Bald Island	53	70	9000
Seal Island	50	1590	190,400
Ledges near Cape Sable Island	46	125	29,000

Certain additional shoals and ledges near Cape Sable Island are among the best of the *Laminaria*-producing regions. No statistics can be given at this time for the amount to be obtained there, for although they were observed and sampled with a Scottish sickle in 1948, grab samples have yet to be made.

TABLE III

SHOWING DENSITIES OF *Laminaria* AND ESTIMATED WEIGHTS IN LESS DENSELY POPULATED REGIONS

Locality	Av. density, lb./sq. yd.	Area, ac.	Estimated weight, tons
St. John Island and adjacent Mainland	11	320	8500
Spectacle Island and Ledges	18	400	17,400
Mud Island	18	250	10,900
Black Ledge	16	50	1900
Lobster I and Ledges	12	150	4300
Gull I and Bar	17	550	22,600
Peggy Island	12	40	1160
Gull Ledge	17	170	7000

A comparison of the density and tonnage of *Laminaria* in southwest Nova Scotia with that of the Orkneys and Outer Hebrides (20, 24) is presented in Table IV.

TABLE IV

Harvestable area	Density	Available tonnage
Orkneys and Outer Hebrides 38,000 ac.	Given density (20), 20 tons per ac.	1,200,000
Yarmouth–Shelburne, N.S. 12,000 ac.	Variation in density, 26-128 tons per ac.	900,000 (sum of weights of individual beds)

The tonnage available in both regions is considered to be a conservative estimate (6).

The greater density in this part of Nova Scotia may be due in part to the high degree of aeration of the coastal waters. Not only are there many tide rips and strong currents, but the Bay of Fundy is noted for having the greatest tidal water exchange in the world (22).

C. *Irish Moss*

Species Occurring in the Survey Area

Gigartina stellata (Stackh.) Batt. (frequently termed "false" Irish moss). In Yarmouth and Shelburne Counties, *Gigartina* is abundant on Black Ledge west of Mud Island and on Devil's Limb and Limb's Limb west of Seal Island. Elsewhere in Yarmouth–Shelburne it occurs rarely and in small amounts. It is found in quantity along Digby Neck, being especially abundant on the Fundy side of the Neck, the density on certain rocks at West Sandy Cove being noted by the writer to range as high as $1\frac{1}{2}$ lb. per sq. ft.

A more detailed report of *Gigartina* will be made on completion of the Digby Neck survey.

Chondrus crispus (L.) Stackh. This species is found generally in a limited zone near low water on all rocky shores throughout the province and for several years has been harvested in Yarmouth and Antigonish Counties. Situated in the mouth of the Fundy tidal region, with its attendant extensive intertidal zone, Yarmouth County shores support a greater growth of harvestable *Chondrus* than most other parts of Nova Scotia.

Extent of Survey

In the area under survey, *Chondrus* is abundant from the islands and ledges near Cape Sable Island, Shelburne County, to Pinkney Point in Yarmouth County, and on the shores in the vicinity of Pembroke.

In Digby County it occurs in the vicinity of Salmon River, and along the eastern shore of St. Mary's Bay from Cape St. Mary's to Meteghan. In this latter situation it grows in practically a pure stand almost unmixed with other algae, and in a more or less continuous belt along the main shore.

In the Bay of Fundy proper, as contrasted with its entrance in Yarmouth County, *Chondrus* is found in small quantities only. Erroneous reports have been made from time to time concerning its abundance in these latter waters. Although it occurs here and there in small quantities on all rocky shores of the Bay there are no large beds known at present. Such erroneous reports as have arisen in that locality have been due to confusion of *Chondrus* with *Gigartina*, which in its purely vegetative condition is sometimes difficult to distinguish from the related *Chondrus*.

Density and Location of Chondrus Beds

Chondrus is harvested from low water to depths of 12 or 14 ft. Although rocks near low water of spring tides yield the greatest density, it occurs also in goodly quantities at depths to $2\frac{1}{2}$ or 3 fathoms, and can be found as deep as 6 fathoms, with negligible amounts at even greater depths. The deeper *Chondrus* usually grows among *Laminaria* spp. and is frequently associated also with *Phyllophora membranifolia*, *Desmarestia aculeata*, *Corallina officinalis*, *Ceramium rubrum*, and other algae.

Density measurements, made in as many accessible locations as possible, were found to vary from 1 lb. per sq. ft. to $2\frac{1}{2}$ lb. per sq. ft., this range being for rocks exposed at low water of spring tides. The average density in such regions was found to be approximately $1\frac{1}{2}$ lb. per sq. ft. The width of the *Chondrus* belt available for rake harvesting from dories averages about 30 ft. at low water springs. The pounds of *Chondrus* stated below were harvested from these lesser depths. Table V shows the amounts harvested in 1950 from the more important beds of the region.

Ecological Conditions and Their Effect on Chondrus Beds

The harvesting grounds listed above represent different types of habitat, and consequently bear different types of plant cover. According to the environment *Chondrus* may grow either in mixed beds where it is closely associated with other algae, or in more or less pure stands. In general, the latter are found in the less sheltered spots, where the surf action and other

TABLE V

AMOUNT OF *Chondrus* HARVESTED AND ITS LOCATION

Pounds harvested	Location of beds
405,141	Cape Sable Island from north of West Head to east of Split Point, and islands and ledges to the south and west as far as Green Point
996,198	Gull Island and western shores of Bon Portage
203,717	St. John Island and St. John Ledge
198,300	Seal Island
965,341	Mud Islands (Mud I., Flat I., Round I., and Noddy I.)
1,318,457	Tusket Islands west of 66° and Old Woman Shoal
18,618	Abbot Island
242,618	West Pubnico Peninsula mainland shore from Abbot Harbour to St. Ann Point, and Twin Islands
3098	Whitehead Island
1296	Ram Island
20,024	Pumpkin Island
64,829	Gull Island
43,943	Lobster Island and Ledges
1154	Snipe Island
2,354,568	Islands and ledges in Lobster Bay, north of 43° 40′, (exclusive of Pumpkin Island and Ram Island) Tusket Islands
47,995	Islands and ledges in Lobster Bay adjacent to Argyle
195,246	Mutton Islands and Ledges in Cockerwit Passage
40,725	Bear Point Thrums
205,387	Shag Harbour and vicinity
779,372	From Chegoggin to Salmon River and from Cape St. Mary's to Meteghan River
7,997,739 (fresh weight)	

conditions are not conducive to extensive growth of *Chaetomorpha melagonium*, or to the more delicate greens such as *Monostroma* spp. Moreover, in exposed situations, the rough water tends to dislodge colonies of hydroids from their substrate and to scatter the reproductive statoblasts of *Electra pilosa* and related bryozoans, which in more sheltered situations easily settle on the stalks of the *Chondrus* where they multiply rapidly and secrete their calcareous encrustation so undesirable to commerce. 'Exposed' *Chondrus* is found on the shores of Cape Sable, Seal Island, Mud Islands, the Tusket Islands,

western shore of Bon Portage, St. John's Ledge, St. John's Island, Rip Point, La Roche, southern ends of the Twins, western and southwest shores of Abbot Island, and on the southeast shores of St. Mary's Bay where there is a minimum of associated weeds.

'Sheltered' *Chondrus*, frequently mixed with other plants, occurs on the Mutton Islands and the nearby mainland and ledges in Cockerwit Passage, on most of the islets and ledges in and near Shag Harbour, in the more land-locked portions of Lobster Bay, and on the sheltered side of many otherwise exposed islands such as the eastern shore of Ellenwood or the northwestern marshy shore of Lobster Island. All these are sheltered situations offering ecological conditions conducive to the growth of encrusting bryozoans and *Lithothamnion* and of *Chaetomorpha* and other shelter-loving algae. In addition, such regions are usually muddy and often support a large population of *Littorina littorea* and *Acmaea testitudinalis*.

Although *Chondrus* from exposed regions is preferred commercially, both exposed and sheltered beds are harvested as is indicated in Table V above. From personal observation and after numerous consultations with workers in the area it is estimated that the total of nearly eight million pounds probably represents about one-third of the available *Chondrus* in this part of Nova Scotia. Present indications are that the 1951 harvest will be larger than those of previous years.

Recolonization of Harvested and Denuded Areas

As the investigation proceeded it became evident that it was necessary to make a study of the effects of various harvesting methods. Accordingly, experiments are being carried out to determine the rate of repopulation after harvesting. Such studies require several years before adequate statistical information can be acquired, and definite conclusions drawn.

Certain conclusive information, however, has been obtained which may be recorded at the present time.

A. *Rockweeds*

Plots have been prepared at different seasons in various ways, and at various levels. Results obtained to date show that on this coast under present harvesting conditions it requires at least three years before full recovery of a harvested *Ascophyllum* area.

In areas where the *Ascophyllum* is cut back too drastically, *Fucus vesiculosus* replaces it to a very large extent. The *Fucus* establishes itself successfully in a shorter period of time than that required for the *Ascophyllum*. It is important therefore, that if an *Ascophyllum* area is to be maintained the plants must not be cut back too drastically nor the area completely denuded, especially as the fruiting season is of short duration, usually lasting for a brief period of two to three weeks in May. The fruiting season of *Fucus vesiculosus* is much more extensive, lasting to some extent throughout the year (11, 12, 14, 15, 21). The details of this work will appear in a separate paper.

Work on the same problem has been carried on in Britain and an account appears in the annual reports of the Scottish Seaweed Research Association (20), and the Journal of the Marine Biological Association of the United Kingdom (11, 26). In general, the results in both countries would seem to be in agreement.

B. *Chondrus*

Experimental areas were prepared in five ways.

(1) Scraping a strip of rock with knives and chisels – strip 3 ft. wide and extending through the *Chondrus* zone, removing basal discs as far as possible with scrapers.

(2) Denuding a similar though narrower strip by means of scraping, followed by chipping away the rock surface.

(3) Shearing as closely as possible with sheep shears.

(4) Raking thoroughly but carefully, removing all branches which could be raked away, yet not injuring the basal discs.

(5) 'Over raking' with consequent damage to basal discs, leaving patches of bare rock.

A sixth area was selected at the same level on the same sloping rock surface. Plants here were measured for length and number per sq. ft. This plot, otherwise left quite untouched by the investigator, was used for comparison with the specially prepared quadrats and is considered for the purpose of the investigation a 'control' area.

Areas (3) and (4) were found to be quite undamaged. After a period of six months for (4) and 18 months for (3) there was no noticeable difference compared with the control area.

Areas (1) and (5), though damaged, are recovering after a period of 20 months.

Area (2), totally freed of basal discs, has supported a successional series of temporary inhabitants, beginning with *Navicula* (*Schizonema*) *Grevillei*, and other attached diatoms. This was followed by delicate and short-lived Chlorophyceae, replaced later in the season by *Scytosiphon* and other brown algae. *Balanus balanoides* appeared in large numbers and remained numerous for about a year. The cover at the time of writing is of *Fucus vesiculosus*, a closed community except for occasional plants of *Chordaria flagelliformis*. Numerous gastropods also inhabit the strip. A similar succesion has occurred on concrete blocks placed in the *Chondrus* bed. Such algal successions are not uncommon and their occurrence is receiving attention elsewhere (13, 16, 19).

Other areas which were severely and 'badly' raked, removing whole clumps of plants with their basal adherent layer, are now becoming filled in with such forms as *Corallina officinalis*, *Lithothamnion spp.*, *Ahnfeltia plicata*, *Cystoclonium purpurea*, and *Chordaria flagelliformis*. In such places gastropods have

increased in number, consuming large quantities of *Chondrus* sporelings and further hindering the rehabilitation of the bed.

That denudation of the rock is disastrous is strikingly shown by the destruction of heavy *Chondrus* beds at St. Ann Point, Pubnico, during the severe winter of 1947-48. Certain rocks at the southern tip of the Point in 1947 were well clothed with *Chondrus*, offering an excellent harvesting ground from which many tons had been raked annually. In the summer of 1948, however, the rocks were observed to carry a heavy covering of the annual *Chordaria flagelliformis*, and bore almost no *Chondrus*. It is presumed that they were denuded of their *Chondrus* by the action of ice, which was unusually heavy during the previous winter. In the autumn of 1949 *Fucus* sporelings were very numerous on these rocks, and by the summer of 1950 *Fucus evanescens* was growing profusely. Except for small amounts in crevices and tide pools, but little *Chondrus* was to be seen. By June 1951, however, the amount of *Chondrus* was noticeably increasing, particularly in cracks and tide pools and on perpendicular rock faces.

The results of these studies are of profound interest even at their present incomplete stage. Not only are the ecological aspects interesting in themselves, but they give information regarding conservation of the beds and should lead to discontinuance of certain faulty methods of harvesting.

C. *Kelps*

Studies are being made on the longevity of laminarias and recolonization of harvested beds.

In the summer of 1948 all visible *Laminaria* sporophytes were cut from an area 80 ft. by 40 ft. east of the Northern Twin Island. By the following spring the patch had recovered sufficiently to make it difficult to locate, though the cover was light and the sporophytes immature. Laminarias continued to increase in number and size until by autumn there was no detectable difference either in general appearance of the area or in weight per square yard as compared with the control area nearby. The weight, however, was somewhat less than that obtained in 1948. The decrease in weight was at first thought to be due to the drastic cutting back of the laminarias, leaving rocks and stones free to roll with the waves. Later observations indicated that the lesser weights were probably due rather to a general variation typical of that season as is illustrated below.

In order to discover, if possible, the incidence of destruction of sporophytes especially in winter, 200 plants were tagged on the Old Woman Shoal in October 1949. In July 1950 only $3\frac{1}{4}\%$ of *L. longicruris* could be found though 15% of the tagged *L. digitata* had survived the winter storms and were in a thriving condition. This high rate of destruction, though seeming excessive, even for such an exposed habitat, would appear to be in keeping with the rate of depopulation of other species elsewhere. Parke (17) found a disappearance of 94–97% of year old sporophytes of *L. saccharina*.

The high mortality rate, particularly evident after winter storms, varies with the species, and with general ecological conditions. There is great variation

too in the number of sporophytes developing in a given bed, and hence in the amount of *Laminaria* available for harvesting. The same beds, which in 1950 were estimated to have a possible yield of 7200 tons, 2400 tons, and 2600 tons, increased their total cover in 1951 to 15,800 tons, 7400 tons, and 8600 tons respectively. The size of the harvest therefore would vary from year to year, the available tonnage being dependent upon prevailing conditions.

In order to check the rate of recolonization and to ascertain the longevity of individual sporophytes, concrete blocks were set out at intervals, beginning in July 1949. The blocks, placed in accessible positions, exhibited a preliminary succession of flora similar to that on blocks placed in *Chondrus* beds. In the summer of 1950 the first *Laminaria* sporophytes appeared. On reaching sufficient size they were tagged with numbered plastic chicken bands and observations are being made on their rate of growth, maturation, and longevity.

The problem of harvesting is somewhat different from that on the Pacific coast where Scagel (18) has recommended harvesting only a portion of the perennial fronds of *Macrocystis*, and portions of the beds of the annual *Nereocystis*. Experimental work now under way must be completed before definite recommendations can be given regarding the best methods of harvesting *Laminaria* in Nova Scotia.

Comparison of Yarmouth–Shelburne and Scottish Surveys

The Scottish Seaweed Research Association has conducted a detailed survey of littoral and sublittoral algae occurring on their coasts. The Scottish waters yield plants of the same general types as those found in the Yarmouth–Shelburne area of Nova Scotia and the records of the two surveys offer an interesting comparison.

The rockweeds exhibit certain specific differences in the two countries, but it is of interest to note that they are always found in greatest profusion under similar circumstances of gentle slope and semishelter, *Ascophyllum* spp. in particular requiring more protection than the others if the plants are to attain a large size (6, 9).

In Nova Scotia *Pelvetia* is absent altogether, and *Fucus serratus* is of extremely limited occurrence in the part of the Province presently under survey. *F. evanescens* and *F. edentatus* are of frequent occurrence here but are not listed in the accounts of the Scottish survey (6, 9, 26). *Ascophyllum nodosum*, though usually having a more limited tidal range than *F. vesiculosus* (9), predominates in both countries (6, 9, 26). In Scotland, both the density of the fucoids and the width of their band is less than in southwest Nova Scotia. As recorded by Gibb (9) the average density for "rich areas" of *Ascophyllum* in Sutherland, Rossshire, and Skye is 16 lb. per sq. yd., and "large gently sloping areas have a width of 26-50 yds." In the Yarmouth–Shelburne area the average density is 37 lb. per sq. yd., and the average width 50 yd. In the Mutton Islands and on the east side of Tusket Wedge the density in some areas reaches 59 lb. per sq. yd. and a width of 150 yd. is not

uncommon. Occasionally, at such places as Abbot Harbour and Forbes Point, the band reaches a width of 250-300 yd. Walker (26) found the highest density, 36 tons per acre, to occur in the Outer Hebrides, while on the east side of Tusket Wedge in Yarmouth County extensive areas of over 100 tons per acre can be harvested. Knight and Parke (11) found *Fucus vesiculosus* in Devon to weigh 14 lb. per sq. yd. In this Province 25 lb. per sq. yd. is quite common in beds of 100% *F. vesiculosus.*

The estimated tonnage of harvestable rockweeds in the Yarmouth–Shelburne survey area is 200,000 tons, occurring on 140 miles (43%) of the 325 miles surveyed. In Scotland, 180,000 tons are found on 540 miles (12%) of the 4250 miles of coast surveyed (26). It is seen, therefore, that not only do the Nova Scotia rockweeds occur in greater density, but in a wider belt, and on a greater percentage of the shoreline.

Without further study it is not possible to make definite comparisons of factors controlling these different densities and widths of zone. Both regions possess a greatly indented coast and waters which are well aerated by strong tidal currents (Scotland 4-7 knots in Shapinsay in the Orkneys (25); Nova Scotia west of Cape Sable Island 4 knots, with many heavy tide rips to the east and west of Lobster Bay). Both have extensive stretches of sand or mud offering no anchorage for holdfasts, and both regions have areas of more solid substratum supporting a rich growth. The slope of the shores at the Fundy entrance and the higher tides found here provide greater width in the intertidal zone, yielding a greater tonnage of rockweed, but this alone should not affect the density. Gibb (9) records Scottish *Ascophyllum* thalli to be 1-6 ft. in length. In the Yarmouth–Shelburne area they are seldom under 3 ft., and frequently are 6-7 ft. In some localities thalli over 10 ft. may be found. Only the younger plants are as short as 1 ft. The greater average length of *Ascophyllum* plants here would indicate greater protection from wave action, and the probability that the degree of shelter more nearly approaches an optimum.

It is less simple to compare the "Irish moss" of the two regions. In Scotland 95% of the crop is *Gigartina*, only 5% being *Chondrus*, while in Yarmouth–Shelburne very little *Gigartina* is found. Marshall, Orr, and Newton (13) estimate the harvestable yield in the Scottish Isles and mainland to be about 380 tons, and give the density variation as $\frac{1}{2}$-2 lb. per sq. yd. The harvest of *Chondrus* in the Nova Scotia survey area for 1950 was almost 4000 tons, and the average density is $1\frac{1}{2}$ lb. per sq. ft. The density of *Gigartina* when found in this area is usually 1 lb. per sq. ft.

The kelps, though differing in species are of the same general types and are found on similar substrata. *Laminaria digitata* is common to both regions and is found on stable rocky bottoms. *L. cloustoni*, so common to Scotland, does not appear in the Nova Scotia waters. *L. saccharina* of the British shores is replaced in Yarmouth–Shelburne by the ecological forms of *L. longicruris* so typical of the region. In both countries these ruffled species are attached to the less stable substrata of stones, mussels and shells, and boulders, as well as

to larger rocks. Moreover, the ruffled laminarias in both countries are found in greatest density in rapid tidal streams.

Both surveys show that density decreases with depth, and that below 6 fathoms but little harvestable weed is found. Under the most favorable conditions in water shallow enough for hand-cutting very heavy densities prevail in both regions: Scotland 70-80 lb. per sq. yd., Yarmouth–Shelburne 60-85 lb. per sq. yd. In the turbid waters of the entrance to the Bay of Fundy, the density decreases noticeably below 4 fathoms, though in lesser depths it is usually greater than that recorded for Scottish areas. The average densities for kelps in the three subareas of the Orkney Island survey as recorded by Walker (25) are: 12.3 lb. per sq. yd., 15.2 lb. per sq. yd., and 16.1 lb. per sq. yd. Chapman (6, 7) quotes the following standards: extremely dense, 20 lb. per sq. yd.; very dense, 15 lb.; dense 12 lb.; moderate 6 lb. The overall average in Scotland is 20 tons per acre. Densities in Nova Scotia were found to vary from 40 to 85 lb. per sq. yd. for weed cut by hand in shallows, and from 4 to 65 lb. per sq. yd. for grab samples cut from 6 to 2 fathoms. In southwestern Nova Scotia the beds as a whole varied in density from 26-128 tons per acre (see Table II).

Table II gives the harvestable area, density, and available tonnage for both regions. The Scottish Islands have 38,000 acres of *Laminaria* beds with 1,200,000 tons. The area surveyed in Yarmouth–Shelburne has 12,000 acres of beds with 900,000 tons.

The limiting factors governing the growth of the three types of seaweeds require further study before a more detailed analysis can be made regarding the contrasts in the two regions. From the data submitted it is evident, however, that the density of rockweeds and Irish moss is greater in the Nova Scotia beds studied than in Scotland, and that greater amounts of these two types of algae are available for harvesting; that the Nova Scotia *Laminaria* spp. are usually denser at least down to four fathoms, but that more may be harvested in Scotland from a larger acreage.

Acknowledgments

The writer wishes to express appreciation to the Scottish Seaweed Research Association for its kindness in supplying blueprints for manufacture of the grab and for advice concerning aerial photography. Acknowledgment is made also to Mr. R. H. Magarvey for his generous help in many ways and especially for advice regarding alteration of the grab for use in Nova Scotia waters; to Prof. S. J. Montgomery for designing the drag, and to Miss Ann Watts for identification of the bryozoan so frequently found encrusting the *Chondrus*. Thanks are due to Dr. A. W. H. Needler for use of the facilities of the Atlantic Biological Station and to Mr. Lloyd Dickie of the Station staff for assistance with the underwater photography. The writer also wishes to express her appreciation to Dr. H. P. Bell, Dalhousie University, for his kindly and encouraging interest in this work. Appreciation is expressed also to the R.C.A.F. for invaluable aid in the aerial photography, and to Kraft Foods, Inc. and to the many Irish moss brokers for assistance in compiling the

Chondrus statistics. The map of the survey area was prepared by Mrs. Helen Stein of the Nova Scotia Research Foundation. Assisting the author during this survey were Messrs. A. M. Shane, D. L. d'Eon, and J. M. Bruce.

References

1. Bell, H. P. and MacFarlane, Constance. The marine algae of the Maritime Provinces of Canada. I. List of species and their distribution and prevalence. Can. J. Research, 9 : 265-279. 1933.
2. Bell, H. P. and MacFarlane, Constance. The marine algae of the Maritime Provinces of Canada. II. A study of their ecology. Can. J. Research, 9 : 280-293. 1933.
3. British Columbia Research Council. Marine plants of economic importance in British Columbia coastal waters. Part I. Tech. Bull. No. 4.
4. British Columbia Research Council. Marine plants of economic importance in British Columbia in coastal waters. Part II. Tech. Bull. No. 10.
5. Cameron, H. L. The use of aerial photography in seaweed surveys. Photogrammetric Eng., pp. 493-501. September 1950.
6. Chapman, V. J. Seaweed resources along the shores of Great Britain. Econ. Botany, 2 : 363-378. 1948.
7. Chapman, V. J. Seaweeds and their uses. Methuen and Co. Ltd., London. 1950.
8. Geological Survey of Canada. Map 10A. 1949.
9. Gibb, Dorothy C. A survey of the commoner fucoid algae on Scottish shores. J. Ecol. 38 (No. 2). November 1950.
10. Goldthwaite, J. W. Physiography of Nova Scotia. Can. Dept. Mines. Geol. Survey Memoir 140, No. 122. 1924.
11. Knight, Margery and Parke, Mary. A biological study of *Fucus vesiculosus* L. and *F. serratus* L. J. Marine Biol. Assoc. United Kingdom, 29 (No. 2) : 439-541. 1950.
12. MacFarlane, Constance and Bell, H. P. Observations on the seasonal changes in the marine algae in the vicinity of Halifax, with particular reference to winter conditions. Proc. Nova Scotian Inst. Sci. 18 (3) : 134-176. 1933.
13. Marshall, S. M., Newton, Lily, and Orr, A. P. A study of certain British seaweeds and their utilization in the preparation of agar. *Edited by* Lily Newton. His Majesty's Stationery Office, London, 1949.
14. Moss, Betty L. Studies in the genus *Fucus*. Ann. Botany, n.s. 12 : 267-279. 1948.
15. Newton, Lily. Handbook of the British seaweeds. The trustees of the British Museum London, 1931.
16. Northcraft, R. D. Marine algal colonization on the Monterey Peninsula, California. Am. J. Botany, 35 : 7, 396-404. 1948.
17. Parke, Mary. Studies on British Laminariaceae. I. Growth in *Laminaria saccharina* (L.) Lamour. J. Marine Biol. Assoc. United Kingdom, 27 : 651-709. 1948.
18. Scagel, Robert F. An investigation on marine plants near Hardy Bay, British Columbia. No. I of a series published at irregular intervals by the Provincial Department of Fisheries, Victoria, British Columbia.
19. Scottish Marine Biological Association, Annual Report 1949-50. Marine algology *by* H. T. Powell. pp. 10-11.
20. Scottish Seaweeds Research Association, Annual Report. 1948.
21. Taylor, W. R. Marine algae of the northwestern coast of North America. University of Michigan, Ann Arbor, Mich. 1937.
22. Tide Tables for Atlantic Coast of Canada. Canadian Hydrographic Service, Dept. Mines Tech. Surveys, Ottawa.
23. Vevers, H. G. Photography of the sea floor. J. Marine Biol. Assoc. United Kingdom, 30 : 101-111. 1951.
24. Walker, F. T. Sublittoral seaweed survey. Part I: Development of the view box spring grab technique for sublittoral weed survey. Part II: Survey of Orkney, Scapa Flow. Part III: Survey of Orkney, Bay of Firth. J. Ecol. 35 (Nos. 1 and 2) : 166-185. 1947.
25. Walker, F. T. Sublittoral seaweed survey of the Orkney Islands. J. Ecol. 38 (No. 1) : 139-165. 1950.
26. Walker, F. T. A seaweed survey of Scotland – Fucaceae. Proc. Linnean Soc. London. 159th Session, 1946-47 (Pt. 2). pp. 90-99. 1947.

GRASSLAND OF THE PEACE RIVER REGION, WESTERN CANADA[1]

By E. H. Moss[2]

Abstract

Natural grassland is associated with poplar and willow groves to form parkland areas on dark soils in the generally forested Peace River region of western Canada. The grassland is described as an *Agropyron–Stipa–Carex* community. Comprising this community are three subtypes or faciations, viz. *Agropyron–Carex* on low areas, *Stipa* on dry slopes, and *Agropyron–Stipa* on mesic sites. For the entire community 154 vascular species are recorded and for the *Agropyron–Stipa* faciation, which is the most common native grassland of the region, 139 vascular species, consisting of 36 graminoids, 84 forbs, and 19 woody species. The leading grasses are *Agropyron trachycaulum*, *Stipa spartea* var. *curtiseta*, and *Koeleria cristata*. The *Agropyron–Stipa* faciation is the "climax" grassland of the region and therefore may be classified as an "association". The occurrence of native grassland areas in the boreal forest region is explained in terms of special physiographic and edaphic features of these areas, notably poorly drained and inadequately aerated soils. Therefore, the *Agropyron–Stipa* grassland may be interpreted as an edaphic climax. Compared with the fescue grassland of south-central Alberta, the Peace River grassland lacks *Festuca scabrella* but has many of the other plants of the fescue association, certain of these assuming the role of leading species. Reliable indicators of early stages in grassland retrogression brought about by heavy grazing are the small sedges, *Carex obtusata* and *C. heliophila*.

Introduction

The occurrence of grassland areas within the forested Peace River region has long been of considerable interest. These areas are associated with aspen poplar and willow groves to form parkland similar to the park or grove belt lying south of the northern or boreal forest and east of the Cordilleran forest in Alberta. They are characterized by dark soils somewhat like the soils of the parkland region to the south. The accompanying map (Fig. 1) shows the dark soils and associated parkland vegetation in relation to the gray wooded soils of the region. The present paper is concerned primarily with a comparison of the grasslands of the Peace River region and those of south-central Alberta and with an attempt to explain the existence of these grasslands and their scattered occurrence in terms of climatic and edaphic factors. Later papers will consider other vegetational features of the Peace River region, including marshy grasslands commonly associated with ponds and lakes.

The most important publications on the vegetation of the Peace River region are those of Raup (9, 10, 11, 12). These papers include catalogues of species reported for the region and excellent discussions on ecological and phytogeographical aspects of the vegetation. The grasslands are described briefly, with particular reference to those of Wood Buffalo Park, and their significance considered in terms of various factors. Raup's papers have

[1] *Manuscript received October 31, 1951.*
Contribution from the Department of Botany, University of Alberta, Edmonton, Alta.

[2] *Professor of Botany, University of Alberta.*

references to early publications on the natural history of the region. Of the more recent papers containing information on grassland may be mentioned that of Groh (2), concerned primarily with weeds, and that of Odynsky and Newton (8) on soils. Of interest also are two unpublished manuscripts, one by Stacey (15) on the native grasses in the southern part of the Peace River region, and one by Marfleet (6), dealing with the sedges of certain grassland areas. Some of Marfleet's data are incorporated in the present paper.

Description of the Region

Only a general account of the physical features, soils, and climate of the region will be given in this section of the paper. Additional information will be presented in connection with a discussion of grassland ecology.

The Peace River region includes a considerable portion of the Alberta Plateau which is that part of the Great Central plain of the Mackenzie basin south and southwest of Great Slave Lake (1). This plateau has a general and very gradual northward slope, the elevation dropping from about 1600 ft. in the south to 1200 ft. in the north. The relatively flat surface of the area as a whole is relieved by several high plateaus or hills which rise 1000 to 2000 ft. above it. Of these, the Caribou Mountains are outstanding, with an altitude of about 3300 ft. and an area of approximately 8000 sq. miles. The main drainage channel traversing the region is the Peace River.

The geological features of the region are presented in papers by Rutherford (13) and others. The soils have been described in several reports and papers by Leahey, Newton, Odynsky, Wyatt, and their colleagues (4, 8, 17, 18, 19). Rock outcrops are infrequent in the area, nearly all of the surface being covered by morainic, fluvial, or lacustrine deposits of recent geological time. The soils derived from the glacial tills and alluvial material are chiefly of the gray, wooded, podsolic kind. There are small areas of somewhat black soils similar to chernozems, and several large areas of degraded soils, transitional in nature between black and gray types. These darker soils, associated as they are with parkland vegetation, will be considered more fully in connection with the vegetational features to be presented.

Data on the climate of the region appear in several publications (5, 8, 9, 10, 15, 16, 17). The climate may be described as continental and dry, with short summers and long, cold winters. The mean annual precipitation varies from about 18 in. at Grande Prairie in the southern part of the region to about 11 in. at Fort Vermilion and northward. In the more northern parts the somewhat shorter growing season, the lower temperature, and the greater proportion of cloudiness compensate for the lower precipitation and make tree growth possible on most kinds of terrain. The relation of climatic factors to the occurrence of natural grassland will be discussed below.

Agricultural development in the region relates closely to the parkland areas, with their native grassland and dark, rich soils, for these areas attracted the early settlers and they still support the bulk of the population of the entire region.

Procedure and Methods

During the course of the grassland studies, the procedure was that of reconnaissance combined with detailed studies of selected samples. Efforts were made to locate areas of native grassland that appeared to have suffered no marked disturbance through man's activities. These efforts were fairly successful, despite extensive settlement and cultivation in the main parklands before the writer began his studies. However, it is worthy of note, that the last two decades have seen a steady reduction in number and size of areas suitable for the investigation of natural grasslands. Heavily grazed grasslands were also studied and compared with relatively undisturbed areas in the same region.

Studies were made on grasslands in the following localities: Grande Prairie, Beaverlodge, Sexsmith, Kleskun Lake, Smoky River, High Prairie, Wanham, Rycroft, Spirit River, Dunvegan, Fairview, Peace River (town), Notikewin, Keg River, Boyer River, Fort Vermilion, Buffalo prairie, south base of Caribou Mountains, Meander River and Fort St. John, B.C. (Fig. 1). In most of these localities, several distinct areas were investigated. The sample areas varied in size from a few square rods to several acres, being commonly about one acre in extent. For each such sample area, records were made of the ecological structure and floristic composition, as well as of any obvious physiographic and edaphic features. Generally, the floristic composition was recorded in the form of a species list and accompanying estimates, subjectively attained, of frequency, abundance, and relative coverage. These estimates are set forth (Tables I–IV), using the following familiar symbols:

d, dominant; *ld*, locally dominant; *a*, abundant; *la*, locally abundant; *f*, frequent or common; *lf*, locally common; *s*, scattered; *o*, occasional; *r*, rare. For several of the areas, quadrat studies were carried out, with a view to quantitative data on species coverage. The quadrats, 9 sq.dm. in size and usually 30 in number were laid out 3 m. apart, across the sample area. For these quadrats the crown cover of each species was estimated and recorded as a percentage of the total area sampled. The results of quadrat studies are summarized in Tables VI and VII. The quadrat data are especially useful in comparing the different types of grassland and in determining the effects of grazing pressure.

The *Agropyron–Stipa–Carex* Community

The grassland areas of this investigation extend over a distance of about 200 miles from south to north and represent quite a wide range of climatic and edaphic conditions. It is not surprising, therefore, that these areas should exhibit considerable diversity in ecological structure and floristic composition. Despite this diversity, however, the entire grassland is regarded by the writer as one ecological category, the *Agropyron-Stipa–Carex* community. Excluded from this community are the very wet grasslands of depressions, characterized by coarse grasses and sedges. These marshy types of vegetation will be dealt

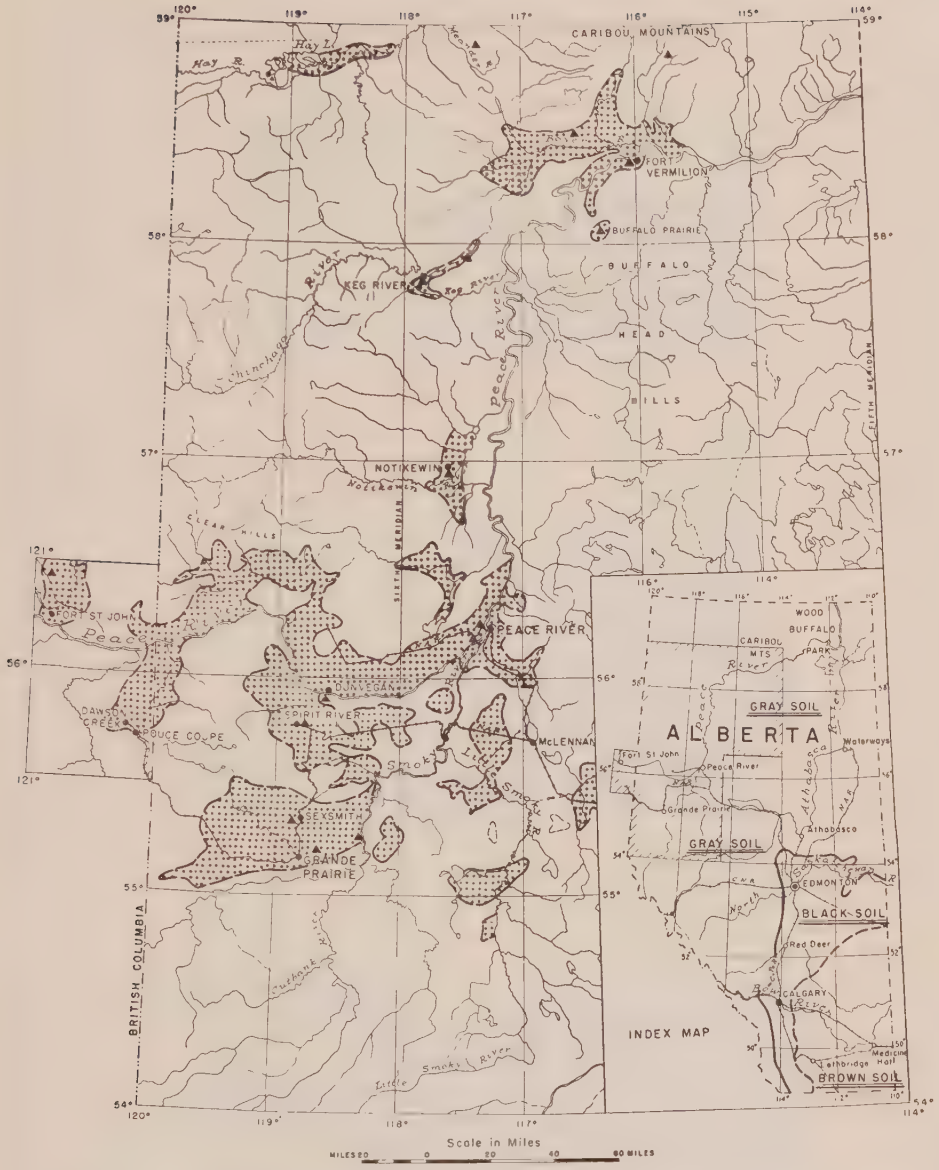


FIG. 1. Outline map of the Peace River region of Alberta and a small portion of British Columbia. The shaded areas, representing parkland vegetation and dark soils, include the natural grasslands of the region. Location of special studies are marked by triangles. On the index map, the broken line marks the transition from *Stipa* prairie of the brown soil zone to *Festuca* grassland of the black soil zone.

with in a later paper. It may be mentioned here, however, that the *Agropyron–Carex* faciation of the *Agropyron-Stipa–Carex* community is believed to follow certain lowland grass–sedge types of vegetation in ecological succession.

Comprising the *Agropyron–Stipa–Carex* community are three subtypes or faciations: (*a*) *Agropyron–Carex*, (*b*) *Agropyron–Stipa*, (*c*) *Stipa* (Tables I–IV). Of these, the *Agropyron–Stipa* faciation is the prevailing one, making up most of the grassland under consideration. It occupies flat, gently undulating, and rolling areas, characterized by mesic grassland habitats (Fig. 4). The *Agropyron–Carex* faciation is confined to low, moist, flat areas (Figs. 2, 3), while the *Stipa* faciation occurs on dry, steep, south-facing slopes of knolls and river valleys (Fig. 5). These faciations intergrade floristically as they do in habitat conditions, the *Agropyron–Stipa* type being climax. Moreover, heavy grazing and drainage tend to change the *Agropyron–Carex* faciation to the *Agropyron–Stipa* type and the latter to the *Stipa* type. For these reasons, the entire grassland is treated as one community.

For the *Agropyron–Stipa–Carex* community, 154 species of vascular plants and a few lichens and mosses have been recorded (Table I). Comprising the vascular species are 29 grasses, 9 sedges, 1 rush, 19 woody plants, and 96 forbs. Though the forbs greatly exceed the other groups in numbers of species, and while some of them are quite prevalent, they are of secondary importance in the association compared with the dominant grasses and sedges. As shown in Table V, some 45 species are recognized as of primary importance in the association, these consisting of 15 grasses, 5 sedges, 6 woody plants, and 19 forbs. It will be noted that only one grass, *Agropyron trachycaulum*, and one shrub, *Symphoricarpos occidentalis*, are rated as leading species for all three faciations, a finding that might suggest a different ecological classification of the grassland. Admittedly, the *Agropyron–Carex* and the *Stipa* faciations are very different. But, as already indicated, each grades into the *Agropyron–Stipa* faciation which is regarded as the theoretical climax type; therefore, they are classified as subtypes of a single community. These faciations will now be considered separately.

Agropyron–Carex Faciation

This grassland was found in only a few locations, the most southerly being near Notikewin. Of the four samples studied, (Table II), only that situated some 30 miles north of Fort Vermilion and east of the Caribou River, near the base of the Caribou Mountains, is regarded as virgin or natural grassland. The area was traversed in passing northward from relatively dry poplar parkland to moist spruce forest at the base of the mountain slope. It appeared to occupy a shallow basin fed by seepage from the heavily wooded mountain slope. As shown in Fig. 2 this grassland is dotted with willow (*Salix Bebbiana*) clumps and flanked by poplar and spruce groves. It is characterized by coarse grasses and sedges, tall forbs, and scattered colonies of shrubs. Certain of the species are locally abundant or dominant, notably *Agropyron trachycaulum*, *Calamagrostis* spp., *Bromus* spp., *Carex atherodes*, *Solidago lepida*, *Vicia americana*, *Symphoricarpos* spp., and *Rosa Woodsii*. Of these, the last three form dense and tangled thickets. The leading species of the faciation are shown in Table V.

FIG. 2. *Agropyron–Carex* grassland with clumps of willow, near south base of the Caribou Mountains.

FIG. 3. Buffalo prairie, north of Buffalo Head Hills; a modified *Agropyron–Carex* grassland.

FIG. 4. Parkland near Peace River town, showing aspen poplar groves on the more moist areas and *Agropyron–Stipa* grassland on the knolls and drier slopes.

FIG. 5. Slopes of river valley near Peace River town, showing parkland with *Stipa* grassland on the drier areas.

FIG. 6. *Stipa* vegetation with the cactus, *Opuntia fragilis*, on a dry slope of the Peace River valley near Dunvegan.

TABLE I

Agropyron–Stipa–Carex COMMUNITY
LIST OF SPECIES RECORDED FOR THE COMMUNITY

(The symbols, denoting frequency and coverage, represent an approximate average of the records for the areas shown in Tables II, III, and IV)

Species	Faciations		*Stipa*
	Agropyron–Carex	*Agropyron–Stipa*	
Grasses, sedges and rushes—			
Agropyron dasystachyum (Hook.) Scribn. (Including *A. riparium* Scribn. & Smith and *A. yukonense* Scribn. & Merr.)		f	f
Agropyron Smithii Rydb.		r	o
Agropyron trachycaulum (Link) Malte var. *glaucum* (Pease & Moore) Malte var. *novae-angliae* (Scribn.) Fern. var. *pilosiglume* (Cassidy) Malte var. *typicum* Fernald var. *unilaterale* (Cassidy) Malte	a	a	s
Agrostis scabra Willd.	o	o	
Avena Hookeri Scribn.	r	o	r
Bromus anomalus Rupr.		r	r
Bromus ciliatus L.	o	r	
Bromus Pumpellianus Scribn.	s	r	
Calamagrostis canadensis (Michx.) Nutt.	o	r	
Calamagrostis inexpansa A. Gray	s	r	
Calamagrostis montanensis Scribn.		r	r
Calamagrostis neglecta (Ehrh.) Gaertn.	r	r	
Carex abbreviata Prescott	r	r	
Carex atherodes Spreng.	a	r	
Carex Deweyana Schwein.		r	
Carex Eleocharis Bailey			r
Carex heliophila Mack.		s	s
Carex obtusata Lilj.	o	f	s
Carex praticola Rydb.	f	s	
Carex siccata Dewey	s	s	
Carex xerantica Bailey		r	r
Danthonia intermedia Vasey		s	r
Elymus innovatus Beal	r	r	
Festuca saximontana Rydb.		r	r
Hierochloë odorata (L.) Beauv.	o	r	
Juncus Vaseyi Engelm.		r	
Koeleria cristata (L.) Pers.	r	f	f
Muhlenbergia Richardsonis (Trin.) Rydb.	r	r	r
Poa ampla Merr.		r	
Poa fendleriana (Steud.) Vasey			r
Poa glaucifolia Scribn. & Williams			r
Poa interior Rydb.	r	o	o
Poa palustris L.	r	r	
Poa pratensis L.	o	o	
Schizachne purpurascens (Torrey) Swallen	s	s	
Stipa columbiana Macoun		o	f
Stipa Richardsoni Link		s	
Stipa spartea Trin. var. *curtiseta* Hitchc.	r	f	a
Stipa viridula Trin.	r	o	s
Shrubs and trees			
Amelanchier alnifolia Nutt.	r	f	s
Arctostaphylos Uva-ursi (L.) Spreng.		r	r
Elaeagnus commutata Bernh.		o	
Juniperus communis L. var. *saxatilis* Pallas		r	

TABLE I—*Continued*

Agropyron–Stipa–Carex COMMUNITY

LIST OF SPECIES RECORDED FOR THE COMMUNITY—*Continued*

(The symbols, denoting frequency and coverage, represent an approximate average of the records for the areas shown in Tables II, III, and IV)—*Continued*

Species	Faciations		*Stipa*
	Agropyron–Carex	*Agropyron–Stipa*	
Shrubs and trees—*Concluded*			
Juniperus horizontalis Moench		r	r
Lonicera glaucescens Rydb.		r	
Populus tremuloides Michx.	o	o	
Prunus virginiana L. var. *demissa* (Nutt.) Torr.	r	o	r
Prunus pennsylvanica L.		r	
Ribes oxyacanthoides L.	r	r	r
Rosa acicularis Lindl.	o	o	
Rosa arkansana Porter		r	s
Rosa Woodsii Lindl.	f	s	
Rubus idaeus L. var. *canadensis* Richards. var. *strigosus* (Michx.) Maxim.	s	r	
Salix Bebbiana Sarg.	o	o	
Salix gracilis Anderss.	o	r	
Shepherdia canadensis (L.) Nutt.		r	
Symphoricarpos albus (L.) Blake	f	o	
Symphoricarpos occidentalis Hook.	f	s	s
Forbs			
Achillea Millefolium L. ssp. *pallidotegula* Boivin	o	s	s
Agastache Foeniculum (Pursh) Ktze.		r	
Agoseris glauca (Pursh) D. Dietr. var. *scorzonaerifolia* (Schrad.) Piper		r	r
Allium cernuum Roth		r	r
Androsace septentrionalis L.		o	r
Anemone canadensis L.	o	r	
Anemone cylindrica A. Gray		r	
Anemone multifida Poir.	r	r	
Antennaria aprica Greene *Antennaria nitida* Greene *Antennaria rosea* (D. C. Eat.) Greene	r	o	o
Antennaria campestris Rydb.	r	r	
Antennaria ? oxyphylla Greene			r
Arabis glabra (L.) Bernh.	r		
Arabis hirsuta (L.) Scop.	r	r	
Arnica Chamissonis Less. (*A. rhizomata* A. Nels.)	r	r	
Arnica fulgens Pursh		r	r
Artemisia camporum Rydb. (*A. caudata* Michx. var. *calvens* Lunell)		r	r
Artemisia frigida Willd.		o	s
Artemisia glauca Pall. var. *dracunculina* (S. Wats.) Fern.	r	o	o
Artemisia ludoviciana Nutt. var. *gnaphalodes* (Nutt.) T. & G.		r	o
Aster ciliolatus Lindl. (*A. Lindleyanus* T. & G.)	r	r	
Aster ericoides L.		r	r
Aster laevis L.	o	o	
Astragalus goniatus Nutt.		r	r
Astragalus striatus Nutt.		r	r

TABLE I—*Continued*

Agropyron–Stipa–Carex COMMUNITY
LIST OF SPECIES RECORDED FOR THE COMMUNITY—*Continued*

(The symbols, denoting frequency and coverage, represent an approximate average of the records for the areas shown in Tables II, III, and IV)—*Continued*

Species	Faciations		*Stipa*
	Agropyron–Carex	*Agropyron–Stipa*	
Forbs—*Continued*			
Astragalus tenellus Pursh			r
Campanula rotundifolia L.	r	o	r
Cerastium arvense L.	r	s	r
Cirsium Drummondii T. & G.	r	r	
Collomia linearis Nutt.	r	r	
Comandra pallida A. DC.		o	s
Delphinium glaucum Wats.	o	r	
Draba nemorosa L. var. *lejocarpa* Lindl.	r	r	
Epilobium angustifolium L.	s	r	
Equisetum arvense L.		r	
Equisetum pratense Ehrh.	r	r	
Equisetum sylvaticum L.	o		
Erigeron caespitosus Nutt.		r	r
Erigeron glabellus Nutt.		o	r
Erigeron strigosus Muhl. (*E. ramosus* (Walt.) BSP.)		r	
Erysimum cheiranthoides L.	r	r	r
Erysimum inconspicuum (S. Wats.) MacM.		r	r
Fragaria glauca (S. Wats.) Rydb.	o	o	r
Gaillardia aristata Pursh		r	
Galium boreale L.	s	s	r
Gentiana Amarella L. (including forma *Michauxiana* Fern.)	r	r	
Geum allepicum Jacq. var. *strictum* (Ait.) Fern.	o	r	
Geum triflorum Pursh		s	s
Hedysarum alpinum L. var. *americanum* Michx.		r	
Heracleum lanatum Michx.	r		
Heuchera Richardsonii R. Br.		r	r
Hieracium umbellatum L.		o	
Lactuca pulchella (Pursh) DC.	r	r	
Lathyrus ochroleucus Hook.	r	r	
Lilium philadelphicum L. var. *andinum* (Nutt.) Ker		r	
Linum Lewisii Pursh		r	r
Lychnis Drummondii (Hook.) S. Wats.		r	
Mertensia paniculata (Ait.) D. Don	s	r	
Monarda fistulosa L. var. *menthaefolia* (Graham) Fern.		r	
Orthocarpus luteus Nutt.		r	o
Opuntia fragilis (Nutt.) Haw.			r
Oxytropis splendens Dougl.		r	r
Parnassia palustris L. var. *neogaea* Fern.	r		
Petasites sagittatus (Pursh) A. Gray	r		
Penstemon gracilis Nutt.		r	r
Penstemon procerus Dougl.		r	r
Polygala Senega L.		r	
Polygonum Douglasii Greene			r
Potentilla arguta Pursh	o	o	r
Potentilla gracilis Dougl. var. *pulcherrima* (Lehm.) Fern. var. *rigida* (Nutt.) S. Wats.	o	r	r

TABLE I—*Concluded*

Agropyron–Stipa–Carex COMMUNITY
LIST OF SPECIES RECORDED FOR THE COMMUNITY—*Concluded*

(The symbols, denoting frequency and coverage, represent an approximate average of the records for the areas shown in Tables II, III, and IV)—*Concluded*

Species	Faciations		*Stipa*
	Agropyron–Carex	*Agropyron–Stipa*	
Forbs—*Concluded*			
Potentilla norvegica L.	o	r	
Potentilla pennsylvanica L.	o	r	
Prenanthes racemosa Michx.		r	
Pulsatilla ludoviciana (Nutt.) Heller		s	f
Ranunculus pedatifidus Sm. var. *cardiophyllus* (Hook.) Britton	r	r	
Rhinanthus groenlandicus Chab.	r	r	
Rubus pubescens Raf.	r		
Senecio cymbalarioides Nutt. var. *borealis* (T. & G.) Greenm.	r	r	r
Senecio pauperculus Michx.	r		
Sisyrinchium angustifolium R. Br.	o	o	
Smilacina stellata (L.) Desf.	r	o	
Solidago decumbens Greene var. *oreophila* (Rydb.) Fern.	r	o	o
Solidago lepida DC. var. *elongata* (Nutt.) Fern. var. *fallax* Fern.	f	r	
Solidago missouriensis Nutt.	r	o	o
Sphaeralcea coccinea (Pursh) Rydb.			r
Stachys palustris L. var. *pilosa* (Nutt.) Fern.	o		
Stellaria longipes Goldie	o	o	
Thalictrum venulosum Trelease	s	s	
Thermopsis rhombifolia (Nutt.) Richards.			r
Vicia americana Muhl.	s	o	
Vicia sparsifolia Nutt.		r	r
Viola adunca J. E. Smith	r	r	
Viola rugulosa Greene	o		
Zigadenus elegans Pursh		r	
Zizia aptera (A. Gray) Fern.	r	r	
Mosses and lichens			
Ceratodon purpureus (Hedw.) Brid.		o	o
Polytrichum spp.	o	o	o
Brachythecium salebrosum (Web. & Mohr.) Bry. Eur.	o	o	
Drepanocladus uncinatus (Hedw.) Warnst.	o	o	
Cladonia spp.		o	o
Peltigera spp.		o	

The other three areas reported in Table II are believed to be somewhat modified forms of the *Agropyron–Carex* faciation. The Keg River prairie developed on a silty flood plain, is characterized by a very fertile black loam, 5 to 10 in. deep, over a brown silt subsoil (19). Most of this prairie has been brought under cultivation within the last two decades. A few relatively undisturbed patches, modified only by moderate grazing and roadside ditching,

TABLE II

Agropyron–Carex FACIATION

(Species are arranged, within the subdivisions, in approximate order of their frequency and coverage)

Species	Typical	Modified		
	South of Caribou Mts.	Boyer River	Keg River	Buffalo Prairie
Grasses and sedges				
Agropyron trachycaulum	a, ld	f, la	f, la	f, la
Carex atherodes	a, ld	f, la	f, la	r, lf
Carex praticola	s, lf	s	f	o, lf
Schizachne purpurascens	f, la	f	r	o
Bromus ciliatus	f, la	s	r	o
Bromus Pumpellianus	s, la	s	s	o
Calamagrostis inexpansa, *Calamagrostis neglecta*, *Calamagrostis canadensis*	s, la	s	o	o
Hierochloë odorata	s	o	s	o
Poa pratensis	o	s	o	s
Agrostis scabra	o	r	s	f, la
Carex obtusata	o	s	s	r
Carex siccata		s	f	f
Koeleria cristata		s		
Elymus innovatus	r	s		r
Poa palustris	r	r		
Poa interior		r	o	r
Avena Hookeri		r		
Stipa spartea var. *curtiseta*		r		
Stipa viridula		r		
Muhlenbergia Richardsonis		r		
Carex xerantica		r		
Carex abbreviata		r		
Shrubs				
Rosa Woodsii	s, la	f, la	s	f, la
Symphoricarpos albus, *Symphoricarpos occidentalis*	s, ld	f	o	s, la
Rubus idaeus (varieties)	o, la	o, la	o	s
Salix Bebbiana	s	o	o	o
Ribes oxyacanthoides	o		o	
Amelanchier alnifolia		f, la		o, lf
Prunus virginiana var. *demissa*		r, la		
Salix gracilis		r	o	r
Populus tremuloides			r	r
Rosa acicularis				s
Forbs				
Vicia americana	a	o	s	o
Galium boreale	s, lf	f	f	s
Solidago lepida	s, la	o, lf	o, la	s
Thalictrum venulosum	s	o, lf	s	s
Epilobium angustifolium	s, lf	o	s	o
Mertensia paniculata	o	s	f	r
Potentilla arguta	o	o	s	s
Delphinium glaucum	o	s	o	o
Fragaria glauca	o	o	o	s
Geum allepicum var. *strictum*	o	o	o	s
Achillea Millefolium ssp. *pallidotegula*	o	o	o	s
Stachys palustris var. *pilosa*	o	o	o	s
Stellaria longipes	o	r	o	r

TABLE II—*Concluded*

Agropyron–Carex FACIATION—*Concluded*

(Species are arranged, within the subdivisions, in approximate order of their frequency and coverage)—*Concluded*

Species	Typical	Modified		
	South of Caribou Mts.	Boyer River	Keg River	Buffalo Prairie
Forbs—*Concluded*				
Anemone canadensis	o	r	o	f
Erysimum cheiranthoides	o	r	r	o
Aster laevis	o	r		s
Viola rugulosa	o	r		s
Collomia linearis	o	r	o	r
Campanula rotundifolia	o	r		o
Cirsium Drummondii	o			
Rubus pubescens	o			
Potentilla gracilis } *Potentilla gracilis* var. *pulcherrima*	r	o	s	s
Sisyrinchium angustifolium	r	r	o	o
Senecio cymbalarioides var. *borealis*	r		r	
Lactuca pulchella	r			
Parnassia palustris	r			
Zizia aptera	r			
Arabis glabra	r			
Petasites sagittata	r			
Potentilla pennsylvanica			s	s
Equisetum sylvaticum			f	s
Heracleum lanatum		r	o	o
Artemisia glauca var. *dracunculina*		o	o	
Potentilla norvegica		o		o
Aster ciliolatus		o		o
Potentilla norvegica		o		o
Smilacina stellata		r	r	o
Viola adunca		r	r	o
Androsace septentrionalis		r		o
Solidago missouriensis		r	r	
Solidago decumbens var. *oreophila*		r		r
Rhinanthus groenlandicus		r		r
Arabis hirsuta		r		r
Lathyrus ochroleucus		r		r
Arnica spp.			r	r
Equisetum pratense			o	
Antennaria spp.			o	
Senecio pauperculus			o	
Lathyrus ochroleucus			o	
Cerastium arvense		o		
Anemone multifida		o		
Gentiana Amarella		r		
Ranunculus pedatifidus var. *cardiophyllus*		r		
Draba nemorosa var. *lejocarpa*		r		

were studied. That the original cover was very similar to the Caribou Mountain grassland is indicated by the present vegetation and by a statement made to the writer by one of the early settlers to the effect that the virgin prairie was a dense growth of coarse "grass" (*Carex atherodes*) and "vetch" (*Vicia americana*). The Boyer River prairie occurs as a narrow strip along

the southern branch of this river, and is similar in origin and development to that of Keg River (19). The more low-lying portions, the subject of the present study, have been moderately grazed and accordingly somewhat altered. The higher and drier parts of this prairie area carry a modified *Agropyron–Stipa* type of grassland. The Buffalo prairie, lying north of the Buffalo Head Hills, seems to have developed on deposits of fine sand and clay, derived from parent material carried down from the Hills (18). The relatively large sample (Fig. 3) of this prairie that was examined showed considerable variation from place to place and provided ample evidence of marked deviation from the original composition. For about 30 years the area has had settlers, who until recently were mainly engaged in stock-raising.

Stipa Faciation

This grassland occurs on dry, south-facing slopes of hills and river valleys, being quite prevalent along the Peace River from eastern British Columbia to Peace River town in Alberta. In Table III are shown records for two localities

TABLE III

Stipa FACIATION

(Species in approximate order of their frequency and coverage)

Species	Peace River (town)	Dunvegan	Smoky River
Grasses and sedges			
Stipa spartea var. *curtiseta*	f, ld	f, la	f, la
Stipa columbiana	s, la	s, ld	s, la
Koeleria cristata	f	s	f
Agropyron dasystachyum	s, la	f, la	f, la
Agropyron trachycaulum	s, la	s, lf	f
Agropyron Smithii		s, la	o, lf
Stipa viridula	s, la	o	s
Poa pratensis	s, la	s, la	o
Carex heliophila	f	s, la	a
Carex obtusata	o	o	f
Poa interior	o	o	o
Avena Hookeri	r	r	o
Calamagrostis montanensis	o	r	
Bromus anomalus			o
Muhlenbergia Richardsonis			o
Danthonia intermedia			r
Festuca saximontana			r
Poa fendleriana		r	
Poa glaucifolia			r
Carex Eleocharis	r		
Shrubs			
Amelanchier alnifolia	s, la	o, la	o
Symphoricarpos occidentalis	s, la	s, la	o
Rosa arkansana	s, lf	s, lf	
Rosa spp.			s
Prunus virginiana var. *demissa*	o, la		o
Juniperus horizontalis		o, ld	
Arctostaphylos uva-ursi			o
Ribes oxyacanthoides		r	

TABLE III—*Concluded*

Stipa FACIATION—*Concluded*

(Species in approximate order of their frequency and coverage)—*Concluded*

Species	Peace River (town)	Dunvegan	Smoky River
Forbs			
Artemisia frigida	s, la	o, la	o, lf
Pulsatilla ludoviciana	s, la	s	o, lf
Geum triflorum	o	s, la	o, lf
Comandra pallida	s	s	s
Achillea Millefolium ssp. *pallidotegula*	s	s	o
Solidago missouriensis	o	o	s
Solidago decumbens var. *oreophila*	f		s
Artemisia glauca var. *dracunculina*	o	s	o
Artemisia ludoviciana var. *gnaphalodes*	o	o	
Orthocarpus luteus	o	o	r
Antennaria aprica *Antennaria nitida* *Antennaria rosea*	o		s
Antennaria ? oxyphylla		o	o
Opuntia fragilis	r, la	r, la	r
Campanula rotundifolia	r	r	o
Senecio cymbalarioides var. *borealis*	o	r	r
Aster ericoides	r		o
Vicia sparsifolia	r	o	
Astragalus striatus		r	r
Polygonum Douglasii		r	r
Astragalus tenellus	r		r
Galium boreale		o	
Erigeron caespitosus		o	
Cerastium arvense			o
Thermopsis rhombifolia			o
Fragaria glauca	o		
Androsace septentrionalis	o		
Penstemon procerus		o	
Oxytropis splendens		o	
Allium cernuum		o	
Arnica fulgens		r	
Artemisia camporum	r		
Penstemon gracilis			r
Erigeron glabellus			r
Agoseris glauca var. *scorzonaerifolia*	r		
Heuchera Richardsonii		r	
Erysimum inconspicuum	r		
Erysimum cheiranthoides		r	
Potentilla gracilis		r	
Sphaeralcea coccinea		r	
Potentilla arguta		r	
Linum Lewisii	r		
Astragalus goniatus			r

on the Peace River and one locality on the Smoky River, northeast of Grande Prairie. The valley slopes (Fig. 5) are characterized by "parkland" vegetation with groves of trees in the more moist situations and with grassland elsewhere, the *Stipa* faciation occurring on the drier exposures. The vegetation is rather sparse, with small patches of bare ground between the tufts of grass and other plants. Striking features of many of the slopes are series of natural contour

terraces produced by landslip. Heavy grazing on some of these slopes has brought about a marked reduction in certain of the grasses and an increase in the sedges and in many of the forbs.

Species of *Stipa* and *Agropyron* characterize this faciation, *Stipa spartea* var. *curtiseta* being the leading grass (Table V). The absence of *Bouteloua gracilis* and *Stipa comata*, common grasses of similar habitats in south-central Alberta, is to be noted. As far as the writer is aware, *Bouteloua gracilis* has not been reported northwest of the Sturgeon River, near Edmonton, and the vicinity of the North Saskatchewan River, northeast of Edmonton. The writer has found *Stipa comata* as far north as Athabasca, 100 miles north of Edmonton. Certain shrubby species are rather common, tending to form colonies of various sizes. The forbs are mainly quite sporadic in their occurrence. The presence of a cactus, *Opuntia fragilis* (Fig. 6), at latitude 56°, is of some interest.

The *Stipa* faciation passes almost imperceptibly into the *Agropyron–Stipa* faciation where the slopes become more gradual or more northward in exposure.

Agropyron–Stipa Faciation (*Association*)

This faciation is by far the most prevalent kind of grassland in the region, occurring on moderately dry slopes (Fig. 4) and on certain flat areas. As already stated, it is regarded as the climax type of grassland for the region; accordingly, it might be given the status of a plant association, a question to be discussed later in this paper.

Actually, this grassland seems often to be so variable in composition as to almost defy analysis or characterization. Some of the variability evidently relates to varying degrees of grazing intensity, some to local edaphic or microclimatic conditions, while other differences may perhaps be explained in terms of the past history of the area and chance introduction of particular species. Despite local variations, this grassland is remarkably constant in its main floristic features throughout the entire region.

The records for the nine areas shown in Table IV are not all of the same value for our main purposes but they do serve to indicate both the variability and the similarity in composition found in different parts of the region. At Fort Vermilion, the sample areas located in level parkland about one mile south of the town are characterized by quite a high proportion of spear grasses. This is probably to be explained in terms of the light textured soil and a very low precipitation (about 11 in.). Quadrat studies (Table VI) show a very high proportion of *Stipa Richardsoni* in a sample area of this grassland. The Boyer prairie, situated on gently undulating land north of the Boyer River and northwest of Fort Vermilion, exhibits a high degree of variability and evidence of rather heavy grazing. At the Meander River, the most northerly area examined, the grassland occurs on rocky ground of an old moraine and on adjoining, well drained alluvial soils (18). Considering the diverse physiographic and edaphic conditions within this area the variability in its vegetation is not particularly great. The Notikewin prairie is a fairly level area with a good, heavy textured, black soil, developed on silty flood plains of streams (19).

TABLE IV

Agropyron–Stipa Faciation

(Species are arranged, within subdivisions, in approximate order of their frequency and coverage)

Species	Fort Vermilion	Boyer Prairie	Meander River	Notikewin	Peace River	Spirit River	Sexsmith	Grande Prairie	Fort St. John, B.C.
Grasses, sedges, and rushes									
Agropyron trachycaulum	f, la	f	f	a	f, la	f, la	f, la	f, la	s, la
Stipa spartea var. *curtiseta*	f, la	f	s	o, la	f, la	f, la	f	f, la	s
Koeleria cristata	f	f	f	o, la	f, la	o, lf	f	f	s
Stipa Richardsoni	f, la	s	o, la	o	o, lf	s	f, la	s, la	o
Schizachne purpurascens	o	s	f, la	f	o	o	s	o	o
Agropyron dasystachyum		o	f, la		s, lf	s, la	f	s	s
Carex obtusata	s	f	o	f	s	s	f	s	s
Carex praticola	o	s	s	f	o	o	s	o	f
Carex siccata	s		f	f	o		s	s	f
Carex heliophila			f		f	f	s	o	f
Danthonia intermedia	r, lf	o	s	o	o, la	o	f	f	
Poa pratensis	s		s	f	r	r	o	s	s, la
Stipa columbiana		o, lf			s, la	o	s		o
Stipa viridula	o		o		o	o, lf			r
Avena Hookeri	o	o	r		r	o	s	o	o
Poa interior			o			o	o	o	o, la
Agrostis scabra	o	r		o	r	r	o	r	
Elymus innovatus		r	o	f					s, la
Calamagrostis inexpansa / *Calamagrostis neglecta*	o	o		r		r	o	r	o
Calamagrostis montanensis					o	o		o	o
Festuca saximontana	r	r	r			r	o	r	o
Carex xerantica	s	r			r	o	s	r	
Carex abbreviata	o	o		r	r	r	r		r
Bromus Pumpellianus	r			f		r			o
Bromus ciliatus		r		r		r	r	r	r
Poa palustris	r			o		r	r		r
Juncus Vaseyi				r		r	r	o	
Hierochloë odorata	r	r						r	o
Calamagrostis canadensis				r					r
Agropyron Smithii						r			o
Bromus anomalus					r	r			
Poa ampla		r						r	
Muhlenbergia Richardsonis		r							
Carex atherodes				r					
Carex Deweyana		r							
Shrubs and trees									
Symphoricarpos occidentalis	s, la	o, la	o, la	f	s, la	o, la	s	o, la	o, la
Rosa Woodsii	s, la	o, la	s	f	o	o	f	o	s, la
Amelanchier alnifolia	s, lf	o, la	o, la	s	s		f	r, la	s, la
Symphoricarpos albus	s, la		o	o			f		s, la
Salix Bebbiana	r	o	o	o		r	r		o
Rosa acicularis		o		s		o	o	o	
Rosa arkansana					s	o	o		
Elaeagnus commutata		o			o, la	r			o
Arctostaphylos uva-ursi			s, la				r	r	r, la
Prunus virginiana var. *demissa*			o, la			r			r
Rubus idaeus (varieties)	r		o	r					
Populus tremuloides	o	r		r					
Ribes oxyacanthoides			r	r					r
Shepherdia canadensis			r						r
Salix gracilis				r			r		
Lonicera glaucescens			r						

TABLE IV—*Continued*

Agropyron–Stipa FACIATION—*Continued*

(Species are arranged, within subdivisions, in approximate order of their frequency and coverage)—*Continued*

Species	Fort Vermilion	Boyer Prairie	Meander River	Notikewin	Peace River	Spirit River	Sexsmith	Grande Prairie	Fort St. John, B.C.
Shrubs and trees—*Conc.*									
Prunus pennsylvanica			r						
Juniperus horizontalis							r		
Juniperus communis var. *saxatilis*						r			
Forbs									
Galium boreale	s	s	f	s	s	s	f	s	f, la
Thalictrum venulosum	o	o	f	f	o	o	f	s	f, la
Geum triflorum	s	f	o	r	s	o	f	o	o
Achillea Millefolium ssp. *pallidotegula*	o	s	o	o	s	r	s	s	o
Aster laevis	o		o	s	o	o	f	o	o
Comandra pallida	o	s	o		s	o	r	o	o
Solidago decumbens var. *oreophila*	r	s	o	o	o	o	s	o	
Pulsatilla ludoviciana		s			f	s	f	o	r
Cerastium arvense		f	f		o	o	r	s	s
Fragaria glauca	o	o	o	o	s	r	o	o	o
Vicia americana	o	r	o	o	o	r	r	o	*s*
Artemisia glauca var. *dracunculina*	o	o	o	o	o	o		r	r
Potentilla arguta	o	s	s	o	r	r	r	o	o
Androsace septentrionalis	o	s	s	r	r	o		r	s
Campanula rotundifolia	o	o	s	o	r	r	o	o	r
Hieracium umbellatum	r	o	o	r	r	r	o	s	r
Viola adunca	o	o	r	o	o	r	r	r	r
Solidago lepida	o	r	o	r		r	s	r	o
Solidago missouriensis		o		o	o	o	r	r	
Stellaria longipes	o	o	o	o			r	o	
Antennaria aprica, *Antennaria nitida*, *Antennaria rosea*	r	s		r	o	r	o	o	o
Smilacina stellata	o	r		o	o	r		r	o
Sisyrinchium angustifolium	o	o		o	r			r	r
Erigeron glabellus	o		r	r		r	s	s	r
Potentilla gracilis (and varieties)			o	o		r	o	r	r
Zizia aptera	r	r		r	r	r	r	r	o
Gentiana Amarella		r	r	r	r		r	o	r
Orthocarpus luteus	o	r		r	r	r		o	
Aster ericoides	r		o	r	r	r	r	r	
Epilobium angustifolium	o	o	o	r		r	r		
Anemone cylindrica	r	o		r	r	r		r	
Potentilla pennsylvanica	o		r		r	r		r	r
Erigeron caespitosus	r	o	r		o			r	
Oxytropis splendens	r	r			r	r			r
Cirsium Drummondii	r		r	r	r	r			r
Delphinium glaucum	r	r	r		s				r
Lathyrus ochroleucus	o		r		r			r	r
Arabis hirsuta	o		r		r	r		r	
Heuchera Richardsonii				r		r	r	r	r
Agoseris glauca var. *scorzonaerifolia*					r	r	r	r	r
Allium cernuum					r	r	r	r	r
Artemisia ludoviciana var. *gnaphalodes*					o	o	r	r	

TABLE IV—*Concluded*

Agropyron–Stipa FACIATION—*Concluded*

(Species are arranged, within subdivisions, in approximate order of their frequency and coverage)—*Concluded*

Species	Fort Vermilion	Boyer Prairie	Meander River	Notikewin	Peace River	Spirit River	Sexsmith	Grande Prairie	Fort St. John, B.C.
Forbs—*Conc.*									
Senecio cymbalarioides var. *borealis*					r	r		o	r
Antennaria campestris		o		r		r		r	
Stachys palustris var. *pilosa*	r		r	o		r			
Aster ciliolatus		o	r	o					
Anemone multifida		r	r			r			r
Hedysarum alpinum var. *americanum*						r	r	r	r
Artemisia camporum		r			r			r	r
Rhinanthus groenlandicus			r		r	r			r
Castilleja ? rhexifolia						r	r	r	r
Astragalus goniatus	o		r					r	
Ranunculus pedatifidus var. *cardiophyllus*						r		r	r
Arnica Chamissonis			r	r				r	
Penstemon procerus				r		r		r	
Prenanthes racemosa	r			r		r			
Erysimum cheiranthoides			r		r		r		
Erysimum inconspicuum	r		r		r				
Collomia linearis	r		r		r				
Vicia sparsifolia						r		o	
Lactuca pulchella	o	r							
Geum allepicum var. *strictum*			o	r					
Gaillardia aristata						r	r		
Lilium philadelphicum var. *andinum*						r		r	
Potentilla norvegica			r	r					
Astragalus striatus	r					r			
Linum Lewisii					r				r
Erigeron strigosus							r	r	
Penstemon gracilis				r	r				
Agastache Foeniculum				r	r				
Anemone canadensis		o							
Equisetum pratense	o								
Lychnis Drummondii								r	
Polygala Senega						r			
Zigadenus elegans								r	
Mertensia paniculata			r						
Arnica fulgens									r
Draba nemorosa var. *lejocarpa*				r					
Monarda fistulosa var. *menthaefolia*									r
Equisetum hyemale	r								
Equisetum arvense	r								
Helianthus laetiflorus var. *subrhomboideus*								r	

The best vegetational records for this prairie were secured by the writer in 1931, near the beginning of extensive agricultural development in the area. Almost no native grassland exists there now. The Peace River records were obtained from an area of rolling parkland (Fig. 4) near the top of the valley,

northwest of Peace River town. The rather rich assortment of grasses and forbs in this area relates to the varied topography. The data for Spirit River were obtained from two slightly disturbed grassland remnants situated on gentle slopes about three miles east of that town. According to Odynsky and Newton (8), the soils of this area are black loam of silt to clay mixture, with a "solodized solonetz type of profile" and developed on lacustrine or alluvial–lacustrine material. The grassland of this area conforms well to the *Agropyron–Stipa* type. The Sexsmith area is situated a few miles southwest of the town and consists of two small portions, one located in a cemetery, the other just outside the cemetery grounds. Both portions of the area seem to have been relatively undisturbed for some time. This grassland is believed to be nearly virgin in composition and representative of the very fertile prairie parkland of the southern Peace River region. The records of Table IV are supplemented by quadrat studies (Tables VI and VII). For Grande Prairie the area studied lies a few miles northeast of the town. It is a fairly large remnant of native prairie on a gentle east-facing slope, and for several years has been used as a winter horse pasture. This area is described as lightly grazed in Tables VI and VII, where a good coverage of grass is recorded. At Fort St. John, B.C., only heavily grazed remnants of the native grassland were found, and the record (Table IV) is for an area of this kind.

As shown in Table IV, the *Agropyron–Stipa* faciation (association) consists of 36 grasses, sedges, and rushes, 84 forbs, and 19 woody plants, a total of 139 vascular species. The data of Tables IV and VI, with accompanying field notes have provided the basis for the list of leading species in the *Agropyron–Stipa* faciation, shown in Table V. Of these species, *Agropyron trachycaulum*, *Stipa spartea* var. *curtiseta*, *Koeleria cristata*, *Carex obtusata*, *Galium boreale*, *Achillea Millefolium*, and *Thalictrum venulosum* may be designated the most constant and characteristic. Others tend to be somewhat localized, though often quite abundant, notably *Stipa Richardsoni*, *Danthonia intermedia*, *Agropyron dasystachyum*, *Carex praticola*, *C. siccata*, and the shrubby species. The sedges make up a considerable portion of the cover of the faciation. Special mention may be made of *Poa pratensis*, a fairly constant and locally abundant species in the grassland, and undoubtedly indigenous to the region.

Indicators of Heavy Grazing

Quadrat studies made during the summers of 1948, 1949, and 1950 were designed in part to clarify our understanding of vegetational changes under grazing by horses and cattle. The results of some of these studies are tabulated in Table VI and summarized in Table VII. These data were supplemented by general observations and by information obtained from farmers as to the usage of particular pastures. The data show that moderately utilized grassland has a crown cover of 62–66% grasses and sedges, 10–12% woody plants, 16–22% forbs, and relatively little bare ground. Under light to moderate grazing the grasses provide 3 to 10 times as much cover as the sedges. Under heavy grazing the grass cover is reduced greatly while the sedge cover increases

TABLE V

Agropyron–Stipa–Carex Community

Faciations

Agropyron–Carex	*Agropyron–Stipa*	*Stipa*
Chief grasses and sedges		
Agropyron trachycaulum	*Agropyron trachycaulum*	*Stipa spartea* var. *curtiseta*
Carex atherodes	*Stipa spartea var. curtiseta*	*Stipa columbiana*
Schizachne purpurascens	*Koeleria cristata.*	*Agropyron dasystachyum*
Bromus ciliatus	*Stipa Richardsoni*	*Koeleria cristata*
Bromus Pumpellianus	*Carex obtusata*	*Carex obtusata*
Carex praticola	*Carex praticola*	*Carex heliophila*
Calamagrostis inexpansa	*Carex siccata*	*Agropyron trachycaulum*
Calamagrostis canadensis	*Danthonia intermedia*	*Stipa viridula*
Hierochloë odorata	*Agropyron dasystachyum*	*Poa* spp.
	Poa spp.	
Leading forbs		
Vicia americana	*Galium boreale*	*Artemisia frigida*
Galium boreale	*Achillea Millefolium*	*Pulsatilla ludoviciana*
Solidago lepida	*Thalictrum venulosum*	*Geum triflorum*
Thalictrum venulosum	*Geum triflorum*	*Comandra pallida*
Epilobium angustifolium	*Solidago decumbens* var.	*Solidago missouriensis*
Mertensia paniculata	*oreophila*	*Solidago decumbens* var.
Potentilla arguta	*Aster laevis*	*oreophila*
Delphinium glaucum	*Vicia americana*	*Artemisia glauca* var.
	Potentilla arguta	*dracunculina*
		Achillea Millefolium
Common shrubs		
Rosa Woodsii	*Symphoricarpos occidentalis*	*Symphoricarpos occidentalis*
Symphoricarpos albus	*Rosa Woodsii*	*Amelanchier alnifolia*
Symphoricarpos occidentalis	*Amelanchier alnifolia*	*Rosa arkansana*
Rubus idaeus (vars.)		

and considerable bare ground appears. Heavy grazing seems to have rather little effect on the total cover of woody plants and of the forbs. But it definitely does alter the proportions of many of the forbs, as may be seen from figures in Table VI.

The most useful of the data pertaining to grazing intensity are those for the two Grande Prairie areas. Located within 300 yd. of each other, on similar soil and terrain and owned by the same farmer, these areas have had quite different treatment over a long period of time. The lightly grazed grassland has been used only as winter pasturage for horses, the heavily grazed area as regular summer pasturage for horses and cattle. The figures in Table VII for these two areas show, under heavy grazing, a reduction in grass cover from 65.2 to 39.8%, an increase in sedge cover from 12.7 to 27.9%, and an increase in bare ground from zero to 13.6%. Two species are involved in the expansion of sedge cover, *Carex obtusata* increasing from 4.6 to 8.8% and *C. heliophila* from 2.7 to 17.0% (Table VI). The figures also indicate that reduction in grass cover is mainly on the part of three species, namely *Agropyron trachycaulum*, *Stipa spartea* var. *curtiseta*, and *S. Richardsoni*, while *Koeleria cristata* increases appreciably under heavy grazing. Though these figures pertain to areas that are not strictly comparable, they may be taken as showing approximately what happens to Peace River grassland under heavy grazing.

TABLE VI

Agropyron–Stipa–Carex Community

Quadrat records for areas exhibiting marked differences in edaphic conditions and in grazing intensity

(Summary of data for coverage in Table VII)

Species	Sexsmith, nearly natural		Grande Prairie, lightly grazed		Grande Prairie, heavily grazed		Fort Vermilion, moderately grazed (upland)		Boyer River, moderately grazed (lowland)	
	Cover	FI*	Cover	FI	Cover	FI	Cover	FI	Cover	FI
Grasses, sedges and rushes										
Agropyron trachycaulum	8.4	57	12.7	73	5.1	67	1.8	13	30.2	97
Stipa spartea var. *curtiseta*	13.8	67	20.6	87	2.8	53	5.9	23	0.7	10
Koeleria cristata	6.0	67	2.3	43	4.4	37	9.4	83	2.3	13
Stipa Richardsoni	8.5	40	4.5	30	0.7	20	42.6	100		
Schizachne purpurascens									2.3	13
Agropyron dasystachyum	3.1	40	1.4	23	1.4	33				
Carex obtusata	4.1	70	4.6	97	8.8	100	1.8	60	1.2	40
Carex praticola	0.8	20					1.5	20	2.8	40
Carex siccata	4.7	30	3.5	60	2.1	30	0.9	17	4.0	47
Carex heliophila	3.7	70	2.7	60	17.0	100				
Danthonia intermedia	4.6	67	21.0	80	21.7	93				
Poa pratensis	0.1	3	0.5	27	0.1	6	0.1	3	1.0	17
Stipa columbiana	1.5	30								
Avena Hookeri	1.1	17			1.2	27				
Poa interior	0.2	6	0.1	10	0.1	6			0.1	3
Agrostis scabra	0.2	6	0.3	20	0.7	27			0.1	3
Elymus innovatus									1.0	27
Calamagrostis neglecta / *Calamagrostis inexpansa*	0.8	6	0.2	3					0.5	6
Calamagrostis montanensis					0.2	13				
Festuca saximontana	0.7	17	1.6	37	1.4	33	0.4	6		
Carex xerantica	1.8	23					1.9	23		
Carex abbreviata	0.3	6					0.1	3		
Bromus Pumpellianus									3.7	17
Bromus ciliatus	0.2	3							4.3	20
Juncus Vaseyi			1.9	30						
Hierochloë odorata									3.3	27
Calamagrostis canadensis									2.7	27
Muhlenbergia Richardsonis									0.3	3
Carex atherodes									1.5	10
Shrubs and trees										
Symphoricarpos occidentalis / *Symphoricarpos albus*	10.9	83					9.2	57	7.4	37
Rosa Woodsii / *Rosa acicularis* / *Rosa arkansana*	1.8	37	1.5	43	0.3	6	1.0	6		
Amelanchier alnifolia	2.3	13								
Rubus idaeus (varieties)									4.8	40
Forbs										
Galium boreale	5.5	100	4.2	90	1.6	53			5.6	60
Thalictrum venulosum	1.5	70	1.3	73			0.3	10	0.8	33
Geum triflorum	1.6	37	0.9	17	1.3	33	4.1	70		
Achillea Millefolium ssp. *pallidotegula*	0.6	53	0.6	53	1.1	33			0.2	13
Aster laevis	2.6	63	2.1	57	0.7	13				
Comandra pallida	0.1	3	0.8	40	2.1	67				
Solidago decumbens var. *oreophila*	0.2	13	1.1	53	3.0	80	4.0	60		
Pulsatilla ludoviciana	1.4	33	0.1	3	0.4	20				
Cerastium arvense			0.2	20	0.7	47	1.1	60		

* *FI (frequency index), percentage of the quadrats in which the species occurred.*

TABLE VI—*Concluded*

Agropyron–Stipa–Carex COMMUNITY—*Concluded*

Quadrat records for areas exhibiting marked differences in edaphic conditions and in grazing intensity—*Concluded*

(Summary of data for coverage in Table VII)—*Concluded*

Species	Sexsmith, nearly natural		Grande Prairie, lightly grazed		Grande Prairie, heavily grazed		Fort Vermilion, moderately grazed (upland)		Boyer River, moderately grazed (lowland)	
	Cover	FI*	Cover	FI	Cover	FI	Cover	FI	Cover	FI
Forbs—*Concluded*										
Fragaria glauca	0.3	27	0.2	17					0.5	6
Vicia americana / *Vicia sparsifolia*	0.3	20	0.3	27	0.1	13			0.6	17
Artemisia glauca var. *dracunculina*							0.8	10		
Potentilla arguta	0.4	27	0.1	6	0.6	13			4.6	60
Androsace septentrionalis					0.6	53	0.6	50	0.1	13
Campanula rotundifolia	0.2	10	0.2	17			0.1	3		
Hieracium umbellatum	0.2	3	0.3	3			1.3	27	0.2	3
Viola adunca	0.1	6	0.7	43	0.2	13			0.1	3
Solidago lepida	0.2	6	0.2	3					0.8	17
Solidago missouriensis			0.2	6	0.1	6				
Stellaria longipes	0.2	10	0.2	17	0.1	13			0.7	37
Antennaria aprica / *Antennaria nitida* / *Antennaria rosea*	0.2	6	0.7	37	0.2	13	0.3	10		
Smilacina stellata							0.7	30	1.3	30
Sisyrinchium angustifolium							0.2	6	0.3	3
Erigeron glabellus	2.6	80	3.0	63	3.3	80				
Potentilla gracilis (and varieties)			0.1	3						
Gentiana Amarella			0.1	3						
Orthocarpus luteus					0.2	20	0.2	20		
Aster ericoides	0.3	6	0.3	17			0.2	6		
Epilobium angustifolium									2.4	40
Anemone cylindrica							0.8	13		
Potentilla pennsylvanica					0.1	6				
Artemisia frigida					0.8	13				
Oxytropis splendens							0.1	3		
Delphinium glaucum									1.6	6
Heuchera Richardsonii	0.2	6	0.1	3						
Agoseris glauca var. *scorzonaerifolia*	0.2	10	0.1	6						
Allium cernuum	0.1	6	0.1	6						
Senecio cymbalarioides var. *borealis*					0.3	13				
Stachys palustris var. *pilosa*									0.6	27
Anemone multifida									0.1	3
Hedysarum alpinum var. *americanum*	1.2	40	0.1	10						
Astragalus goniatus			0.1	6						
Ranunculus pedatifidus var. *cardiophyllus*									0.1	3
Erysimum inconspicuum	0.1	3								
Collomia linearis									0.1	3
Lactuca pulchella							0.9	20		
Geum allepicum var. *strictum*									0.3	6
Gaillardia aristata	0.3	10			0.3	6				
Lilium philadelphicum var. *andinum*			0.1	3						
Astragalus striatus							0.3	13		
Anemone canadensis									0.7	13
Mertensia paniculata									0.6	27
Helianthus laetiflorus var. *subrhomboideus*			0.1	3	0.1	6				
Mosses and lichens			2.0	77	0.5	6	0.2	3	0.8	23
Bare ground					13.6	67	7.2	83	2.5	30

* *FI (frequency index), percentage of the quadrats in which the species occurred.*

TABLE VII

SUMMARY OF COVERAGE DATA SHOWN IN TABLE VI

Percentage of crown cover for different groups of species, as affected by varying conditions

	Sexsmith, nearly natural	Grande Prairie, lightly grazed	Grande Prairie, heavily grazed	Fort Vermilion, moderately grazed (upland)	Boyer River, moderately grazed (lowland)
Grasses	49.2	65.2	39.8	60.2	50.5
Sedges	15.4	12.7	27.9	6.2	11.5
Grasses and sedges	64.6	77.9	67.7	66.4	62.0
Shrubs and trees	15.0	1.5	0.3	10.2	12.2
Forbs	20.5	18.7	17.9	16.0	22.3
Mosses and lichens	—	2.0	0.5	0.2	0.8
Bare ground	—	—	13.6	7.2	2.5

The foregoing conclusions regarding sedges, based on Table VI, are supported by other data for the Peace River region and by records for the *Festuca scabrella* grassland of southwestern Alberta secured by Marfleet (6). The most important conclusions reached by Marfleet, for both the Peace River region and southwestern Alberta, are that *Carex obtusata* and *C. heliophila* increase appreciably even under moderate grazing, and that these sedges may be used as indicators of early stages in retrogression of native grassland, even in advance of such well known indicators as *Artemisia frigida.*

Discussion

Ecological Relationships and Climax Grassland

The *Agropyron–Carex* faciation has evidently succeeded a marsh community characterized by coarse grasses and sedges, including *Calamagrostis inexpansa* and *Carex atherodes.* These prominent members of the *Agropyron–Carex* faciation may be interpreted as relict species that have persisted from the lower community. Upon further drying of the lowland areas, the *Agropyron–Carex* faciation develops into the *Agropyron–Stipa* faciation, which is believed to be the grassland of mesic habitats and the climax type. The *Stipa* faciation, the grassland of xeric areas in the region, is succeeded by the *Agropyron–Stipa* faciation, when these areas become transformed into mesic habitats, as may occur by gradual leveling of the terrain through the course of time. Thus the *Agropyron–Stipa* faciation is at least a theoretical climax to the *Stipa* type. Therefore, the *Agropyron–Stipa* faciation may be regarded as the climax grassland of the region and, accordingly, might be given the rank of a plant association.

However, if the category of "association" be reserved for the highest or final kind of vegetation that will develop under the prevailing climate, there would appear to be some doubt about classifying the Peace River grassland as such. Considerable evidence points to this grassland as being an edaphic climax rather than a true climax (in the Clementsian sense); therefore, the rank of "association" is assigned with some hesitation.

Evidence that the *Agropyron–Stipa* grassland is not the true or climatic climax vegetation, but rather an edaphic climax, will be considered briefly. As will be brought out in a later paper, there is a marked tendency for willow thickets to establish and to persist in the grassland and for poplar woods to encroach upon this grassland. Eventually, barring a major change in climate, most of the grassland areas are destined to become wooded. But it seems that the change from grassland to forest has long been effectively retarded by fires and by certain edaphic factors. Biotic influents such as bison and domesticated animals are considered to be only locally effective in preventing the advance of trees. Soper (14) is probably correct in his view that the buffalo (bison) of the region did relatively little damage to woodland growth, pointing to the conclusion that this influent may be disregarded in considering the question of grassland persistence.

As in other parkland regions of Alberta, repeated burning has doubtless been a major factor in counteracting the tendency of trees to invade the grassland. This has happened in various parts of the country, including the region north and west of Fort Vermilion, where a number of prairie species, notably *Danthonia intermedia*, *Stipa spartea* v. *curtiseta* and *Koeleria cristata*, are associated with woodland shrubs and herbs in a mixed vegetation. It may be emphasized here that man is chiefly responsible for the fires that have brought about this retrogression to grassland.

Of considerable significance in connection with the persistence of the grassland areas through long periods of time are certain edaphic factors. Brief mention may be made here of the relatively stable xeric conditions that tend to maintain the *Stipa* faciation on very dry areas. On moderately dry slopes, the physiography is such as to favor the *Agropyron–Stipa* grassland in competition with poplar vegetation. The poplar root suckers extend but slowly into the grassland, and any gain that may be made by the poplar is likely soon to be offset by the occasional fire or by certain animal influents, especially rabbits and grazing animals that cause damage to the young trees. On the gently undulating and flat grassland areas, there are other significant edaphic factors. Certain of these areas, e.g. Fort Vermilion, have light soils which, under the prevailing dry climate, seem to favor a parkland with grass patches and poplar groves almost in equilibrium, but which permit a shift to grassland under repeated burning. Lowland areas, e.g. Keg River and Buffalo Prairie, evidently have persisted as grasslands because their heavy, poorly aerated, alluvial soils, as well as their tight sod and dense plant cover, have militated against the success of trees; moreover, periodic flooding may have brought about the killing of trees that have become established. At least some of the

undulating areas are known to have heavy soils and special features of these soils that seem to relate to the persistence of grassland. Of these, the area east of Spirit River has been described as having parent soil material from marine shales and a solonetz type of soil profile which is characterized by a very hard impervious B horizon or "clay pan" relatively near the surface (8). As Odynsky and Newton suggest, the clay pan may be unfavorable for tree growth and so may account for the occurrence of grassland on these areas during a long period of time and the production of dark colored soils.

Comparison with Other Alberta Grasslands

The description of Peace River grassland presented in this paper amplifies the general account given by Raup (9) for the same region and supports Raup's description of prairies lying north of the lower Peace in Wood Buffalo Park (10). The latter prairies are only 50 to 150 miles northeast of the Caribou Mountain and Fort Vermilion areas reported in the present paper. It is not surprising, therefore, that the *Agropyron–Stipa–Carex* grassland should bear a close resemblance to the prairies described by Raup for Wood Buffalo Park. Especially striking is the similarity between the *Agropyron–Carex* faciation of this paper and the low grassland described by Raup for areas close to the east base of the Caribou Mountains. Most of the 43 species listed by Raup for two samples of this grassland are rated as leading species in the *Agropyron–Carex* faciation. The drier grasslands of Wood Buffalo Park, as described by Raup, resemble closely the *Agropyron–Stipa* faciation of the present paper. The commonest variations from the two main types in Wood Buffalo Park relate to saline deposits and have a halophytic element. Similar saline vegetation has been seen by the writer near Fort Vermilion and will be reported in a later paper. It may be concluded, therefore, that the same grassland types (those comprising the *Agropyron–Stipa–Carex* community) occur throughout the entire Peace River region of Alberta and northward in wood Buffalo Park on the west side of Slave River. As Raup has pointed out, the relationship between these grasslands and those of the arctic tundra constitutes "one of the most intriguing problems in the north" (11).

The Peace River grassland has rather close floristic affinities with the fescue grassland as described (7) for south-central and southwestern Alberta, but differs in one outstanding respect, viz., the absence of *Festuca scabrella*, which is the dominant grass of the fescue association. It differs also in other features to be indicated below. An appropriate comparison may be made between the *Agropyron–Stipa* faciation (the climax "association") of the Peace River region and the *Festuca scabrella* association of the more southern region. Most of the cover of the *Agropyron–Stipa* grassland is made up of grasses that are secondary in the *Festuca scabrella* grassland, notably, *Agropyron trachycaulum*, *Koeleria cristata*, and *Stipa spartea* var. *curtiseta*. Incidentally, these species become prominent in the fescue grassland as the fescue decreases under heavy grazing. Of 139 species recorded in this paper for the *Agropyron–Stipa* faciation and 148 species recorded (7) for the *Festuca scabrella* association, 77 species are common to both, 62 occur in the first but not in the second, and

71 occur in the second but not in the first of these grasslands. Considering the graminoids, 15 species are common to both, while 21 species found in the *Agropyron–Stipa* faciation are absent from the *Festuca scabrella* association. Included among these 21 species are several sedges (notably *Carex praticola* and *C. siccata*) and grasses that in the more southern parts of Alberta belong to lowlands and woodlands (e.g. *Poa palustris*, *Bromus Pumpellianus*, *B. ciliatus*, *Schizachne purpurascens*). A similar comparison for the forbs shows 55 species common to both grasslands, while 29 species found in the *Agropyron–Stipa* faciation are absent from the *Festuca scabrella* association. Here again the northern grassland has many species which farther south are characteristic not of prairie, but of somewhat marshy habitats or of woodland, e.g. *Stachys palustris* var. *pilosa*, *Equisetum* spp., *Delphinium glaucum*, *Epilobium angustifolium*, *Lathyrus ochroleucus*, *Vicia americana*. There are, therefore, in the northern prairies, several grasses and forbs which farther south are restricted to more moist substrata. Presumably, this relates to the lower evaporation rate and somewhat shorter growing season of the north, but an adequate explanation awaits further investigation. In the *Festuca scabrella* association there are 8 graminoids, 3 woody species, and 60 forbs not reported for the *Agropyron–Stipa* consociation. Most of these species are found only in the more southern part of the fescue grassland and are believed to be migrants from the south and west (7).

In passing, it may be mentioned that there is one record of *Festuca scabrella* for the Peace River region. This species was found by the writer, as two small patches, close together and each about three feet across, in seminatural *Agropyron–Stipa* grassland, near a main highway, about 10 miles east of Grande Prairie. It seems almost certain that this occurrence of the grass is to be explained as a fairly recent chance introduction. Settlers from the south may have brought it to the area as seed in wild hay ("prairie wool"), probably carried in their wagons as packing or as feed for their horses. This record for *F. scabrella* suggests speculations as to how rapidly this grass would extend in Peace River prairies, whether it is to be regarded as a potential dominant there and whether a fescue association would ultimately be developed under natural conditions.

Phytogeographical Considerations

The earlier discussion pertaining to climax vegetation assumes a long period characterized by present climatic conditions. The dark grassland soils provide evidence of fairly constant conditions of long duration. The fact that many of the dark soil areas are now wooded and show the degrading influence of tree cover might suggest a rather recent change in climate. But, this transformation may be explained apart from any marked climatic shift. For these areas, long under edaphic control, have evidently become so altered that they are now subject to the master control of climate and show succession to the woodland climax. It is probable, however, that some of these transition areas have had grassland and woodland alternately through a long period, these changes corresponding to relatively minor fluctuations in climate.

The possibility of major climatic shifts for the region during postglacial time is worthy of some consideration, especially the suggestion that the present climate was preceded by a prolonged warm-dry (xerothermic) period when grassland was much more extensive than at present. On this hypothesis, the existing prairie patches, especially the drier of these, would be regarded as remnants of a very extensive grassland which occupied much of the region during the xerothermic period and which might have been connected with grassland of south-central Alberta. The numerous floristic elements common to the Peace River and more southern prairies might well be taken to indicate a community of origin for these grasslands and to provide support for the xerothermic hypothesis. This hypothesis finds considerable support in the recent work by Hansen on pollen analysis of peat sections in Alberta. Hansen (3) suggests that the Peace River grasslands may be relicts of a postglacial expansion of prairie "during a warm, dry maximum between 8,000 and 4,000 years ago". On the other hand, Raup (9, 10) sees no need to postulate a warm-dry segment of time for the boreal region and envisages only a gradual amelioration of the climate since glaciation. He stresses the probability of a fairly short postglacial period for the introduction and establishment of vegetation in the region. During this period, Peace River grasslands may have developed from tundra through subarctic grass–sedge stages. The strong *Carex* element in the Peace River grasslands may be significant in this connection. These interrelated phytogeographical problems will doubtless be clarified through further study of peat profiles and of subarctic "prairies".

Acknowledgments

The writer's investigation of Peace River grassland commenced in 1931, when he was ably assisted by his wife in rather extensive studies. Between 1931 and 1947 occasional brief visits were made to the region and some useful data obtained. During portions of four summers, 1947 to 1950, an ecological survey sponsored by Science Service, Dominion Department of Agriculture, Ottawa, was conducted in accordance with the general instructions of the Division of Botany and Plant Pathology. For the facilities extended, grateful acknowledgment is made to the Director of Science Service, Dr. K. W. Neatby, and to the Dominion Botanist, Dr. J. H. Craigie. For valuable advice and for assistance with determinations of specimens, appreciation is expressed to Dr. H. A. Senn. Indebtedness is also acknowledged to Dr. B. Boivin, Dr. R. F. Cain, and Mr. A. Breitung for making determinations. During the survey, the writer was assisted in the field by Dr. C. Frankton, Dr. D. A Fraser, and the following students: Messrs. R. L. Airth, F. C. Marfleet, D. A Lang, and J. B. Millar. Facilities provided by Mr. V. J. Lowe, Officer-in-Charge, Dominion Experimental Substation, Fort Vermilion, made possible a pack-train journey to the Caribou Mountains. The writer is indebted to Mr. William Odynsky and Dr. J. D. Newton for information on the soils of the

region, to Mr. J. Groot, Research Council of Alberta, for making the map used in this paper, and to my colleague, Miss Lorene Kennedy, for assistance with the photographs.

References

1. CAMSELL, CHARLES and MALCOLM, WYATT. The Mackenzie River Basin. Can. Geol. Survey Mem. 108. 1921.
2. GROH, HERBERT. Plants of clearing and trail between Peace River and Fort Vermilion, Alberta. Can. Field Naturalist, 63 : 119-134. 1939.
3. HANSEN, HENRY P. Postglacial forests in south central Alberta, Canada. Am. J. Botany, 36 : 54-65. 1949.
4. LEAHEY, ALFRED. Leaching of mineral matter in some Alberta soils. Sci. Agr. 13 : 7-15. 1932.
5. LOWE, VICTOR J. Fort Vermilion, Dominion Experimental Substation, Progress Report, 1939–1948. 1950.
6. MARFLEET, F. C. Taxonomy and ecology of grassland sedges. Unpublished thesis, University of Alberta. 1950.
7. MOSS, E. H. and CAMPBELL, J. A. The fescue grassland of Alberta. Can. J. Research, C, 25 : 209-227. 1947.
8. ODYNSKY, W. and NEWTON, J. D. Soil survey of the Rycroft and Watino Sheets. Research Council of Alberta. Rept. No. 56. University of Alberta. Bull. No. 53. 1950.
9. RAUP, H. M. Phytogeographic studies in the Peace and Upper Liard River regions, Canada. Contribs. Arnold Arboretum (Harvard Univ.), No. 6. 1934.
10. RAUP, H. M. Botanical investigations in the Wood Buffalo Park. Natl. Museum Can. Bull. No. 74. 1935.
11. RAUP, H. M. Botanical problems in boreal America. Botan. Rev. 7 : 147-248. 1941.
12. RAUP, H. M. Additions to a catalogue of the vascular plants of the Peace and Upper Liard River regions. J. Arnold Arboretum (Harvard Univ.), 23 : 1-28. 1942.
13. RUTHERFORD, RALPH L. Geology and water resources in parts of the Peace River and Grande Prairie districts, Alberta. Research Council of Alberta, Rept. No. 21. 1930.
14. SOPER, J. DEWEY. History, range and home life of the northern bison. Ecol. Monographs, 11 : 349-412. 1941.
15. STACEY, E. C. Ecological studies on the native grasses of the Grande Prairie–Beaverlodge district. Unpublished thesis, University of Alberta. 1927.
16. STACEY, E. C. Climatic factors affecting crop production. Sci. Agr. 26 : 79-82. 1946.
17. WYATT, F. A. Preliminary soil survey of the Peace River–High Prairie–Sturgeon Lake area. Research Council of Alberta. Rept. No. 31. 1935.
18. WYATT, F. A., DOUGHTY, J. L., and YOUNGE, O. R. Preliminary soil survey of Fort Vermilion district. Unpublished manuscript. Research Council of Alberta. 1930.
19. WYATT, F. A. and LEAHEY, A. Report of a preliminary soil survey of the area from Grimshaw to Keg River between the sixth meridian and the Peace River. Unpublished manuscript. University of Alberta. 1929.